Medical Library

JUN 3 1946

Volume 38 Number 4

ENDOCRINOLOGY

APRIL, 1946

ALBERT, A., AND WILLIAM L. MONEY

The Comparative Effect of Human Chorionic and Equine Gonadotrophins on the Rate of Ovarian Growth 219

DANOWSKI, T. S., E. B. MAN AND A. W. WINKLER

Tolerance of Normal, of Thyroidectomized, and of Thiourea or Thiouracil Treated Dogs to Oral Desiccated Thyroid and to Intravenous Thyroxine. 230

REED, C. I., B. P. REED AND W. U. GARDNER

The Influence of Estrogens on the Crystal Structure of Bone as Revealed by X-Ray Diffraction Studies on the Femora of Mice 238

CANZANELLI, ATTILIO, RUTH GUILD AND DAVID RAPPORT

The Effects of Alloxan Diabetes on the In-Vitro Oxidation of Substrates by Rat Tissues 245

ZECKWER, ISOLDE T.

The Passage of Endogenous Estrogen Across the Parabiotic Union in Rats 249

NOTES AND COMMENTS

GREISHEIMER, ESTHER M., M. J. OPPENHEIMER AND DOROTHY ELLIS

Acid-Base Equilibrium in Relation to the Menstrual Cycle 258

BENUA, RICHARD S., AND EVELYN HOWARD

A Note on the Absence of Androgen from a Diet of Natural Foods 260

RAPPORT, DAVID, ATTILIO CANZANELLI AND RUTH GUILD

The Effect of Thyroidectomy on the Tissue Response to Administered Thyroxin 260

PUBLISHED MONTHLY FOR
THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

Annual Subscription, $10 50 Single Copies $1.25

Cryptorchidism

Undescended testes are exposed to several hazards —atrophy, sterility, torsion and malignant degeneration. Even if these unfortunate complications do not eventuate, the very existence of cryptorchidism may be responsible for or contribute toward psychic disturbances in adolescence.

PRANTURON

Administration of PRANTURON, chorionic gonadotrophin, at an early age often causes the cryptorchid organ to assume its natural position and thereby promotes somatic and emotional readjustment.

PRANTURON is available in two strengths as a stable powder in multiple dose vials to permit flexibility in dosage, each vial containing 5,000 I.U. or 10,000 I.U. of dry, highly purified hormone of pregnancy urine. When diluted with the sterile diluent provided, solutions of 500 I.U. per cc. or 1,000 I.U. per cc. are obtained. Packaged in boxes of one vial.

Trade-Mark PRANTURON—Reg. U.S. Pat. Off.

SCHERING

Schering CORPORATION · BLOOMFIELD · N. J.
In Canada, Schering Corporation Ltd., Montreal

ENDOCRINOLOGY

VOLUME 38 APRIL, 1946 NUMBER 4

THE COMPARATIVE EFFECT OF HUMAN CHORIONIC AND EQUINE GONADOTROPHINS ON THE RATE OF OVARIAN GROWTH[1]

A. ALBERT AND WILLIAM L. MONEY

From the Biological Laboratories, Harvard University

CAMBRIDGE, MASSACHUSETTS

FEW STUDIES have been made on the kinetics of ovarian growth under the influence of gonadotrophic hormones. Most quantitative investigations have been concerned with methods of bioassay, or with the interaction and antagonism of various gonad stimulating hormones. The technique employed in such problems has been to inject the hormone once or many times daily over several days and to weigh the ovaries or some other organ as end point. By using increasingly larger doses in this manner, the comparative potency of various gonadotrophins has been determined.

It seemed to us that significant additional information might be obtained by a study of the rate of ovarian response to gonad stimulating hormones. Furthermore, it might be found that each gonadotrophin induces a characteristic rate of ovarian growth. If this proved to be the case, then new and simpler methods of differentiation among gonadotrophins might be worked out, and mixtures of gonadotrophins possibly resolved.

However, the determination of rate of response is beset with considerable difficulty since there is no convenient way of attaining, or determining, a constant known blood level of a gonadotrophin by the conventional method of multiple daily injections. If gonad stimulating hormones were effective after but one injection, the rate of response could be calculated from suitably constructed time-effect curves.

Received for publication February 8, 1946.

[1] Aided by a grant from the Milton Fund to Professor F. L. Hisaw.

Copyright, 1946, by the Association for the Study of Internal Secretions.

That a single injection of a gonad-stimulating hormone such as found in the serum of pregnant mares (PMS) could induce a marked increase in ovarian weight was first indicated by Cole, Guilbert and Gross (1932). Further work has shown that PMS is nearly as effective on the immature rat ovary when administered subcutaneously in a single injection as when the same total dose is given in multiple injections over many days (Evans, Meyer and Simpson, 1933; Catchpole and Lyons, 1934; Cole, 1936; Hamburger and Pedersen-Bjergaad, 1938; Leathem, 1941). For PMS, at least, the efficacy of a single injection is such that a convenient assay has been developed by Cole and Erway (1941) using a single injection of hormone and recording ovarian weights 48 hours later.

Because of this property, and because PMS is beyond reasonable doubt a single hormone, we endeavored to determine its effect on the rate of ovarian growth of immature rats. For purposes of comparison, we chose the chorionic gonadotrophin in human pregnancy urine (PU) because it is likewise a single substance, however, of lesser ovarian stimulating ability.

MATERIALS

Three different preparations of PMS were used: (1) sterile serum of pregnant mares,[2] #29; (2) the International Standard preparation; and (3) a commercial concentrate, Antex-Leo. Two preparations of PU were used, one a commercial concentrate, Follutein, prepared by E. R. Squibb and Sons, and the other, a sample of International Standard preparation.

About 1800 female rates, 21 days of age, were used for this study, two thirds being used for PMS, and one third for PU. Because of other demands on our colony, experiments were done at various intervals over the past 4 years.

RESULTS

1. Pregnant mare serum

In order to study the response of the ovary to single injections of varying amounts of pregnant mare serum (#29) we injected groups of 30 animals with doses of serum ranging from 0.05 cc. to 1.0 cc. per animal. Wherever necessary dilutions of PMS were made in saline so that no animal received less than 0.5 cc. of fluid. Each rat was injected subcutaneously in the back of the neck since Leathem (1941) had shown that considerable individual variation resulted with intraperitoneal injections. Groups of 5 animals were killed at 24-hour intervals over six days, and the ovaries weighed. The data from these experiments are shown in Figure 1, where 5 different dosages of PMS were tested. It is seen that there are small differences in ovarian weights 24 hours following a single injection of various amounts of serum. At 48

[2] This serum was kindly furnished to us by Dr. H. H. Cole who stated that it contained 25 Cole rat units per cc. The serum (#29) was collected from a Percheron mare at the 79th day of pregnancy.

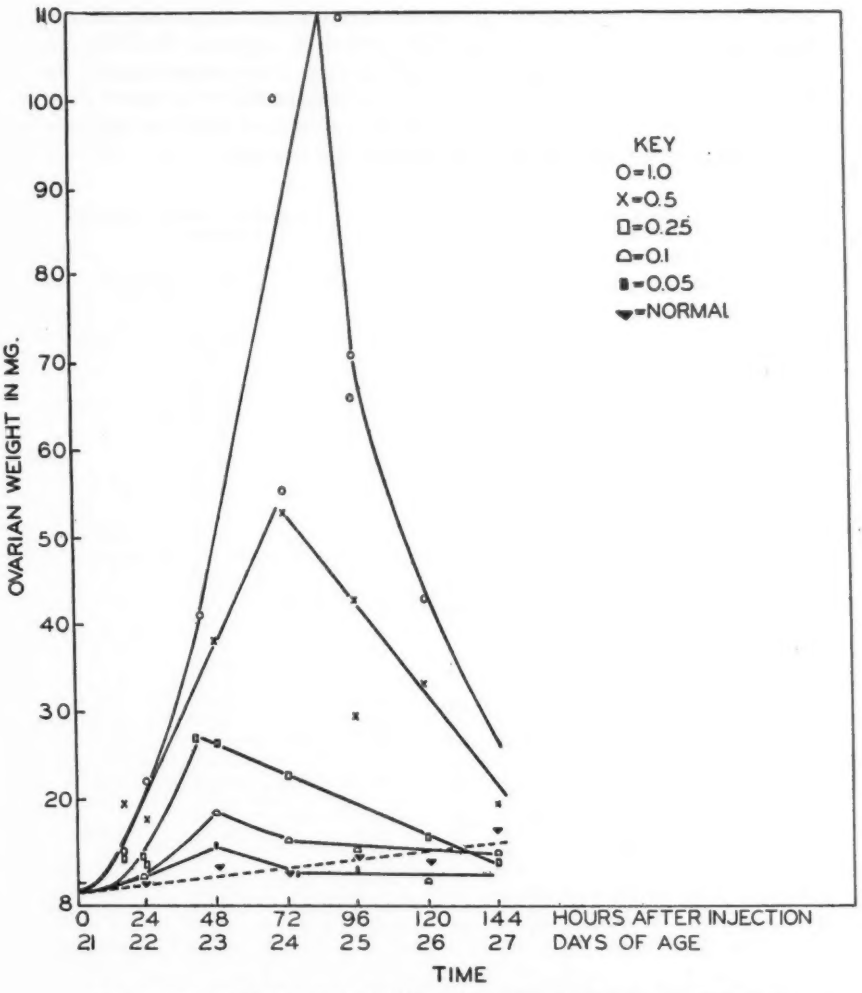


FIG. 1. The effect of a single injection of pregnant mare serum (#29) on the response of the ovary at 24-hour intervals following injection. Normal ovarian weights of our colony shown in dotted line, 20 animals per point, total 120 animals. All other points consist of 4–5 animals each, total about 170 animals.

hours, however, these differences are much larger. Regression in the ovarian weight occurs after 48 hours. The time at which regression occurs is directly proportional to the amount of serum given; in the 2 larger doses, regression occurs 72 and 96 hours following injections. Examination of the curves in Figure 1 shows that growth rates can be determined between 24 and 48 hours following an injection since

good increases in ovarian weight occur only during this period. Where there are several points during this period, it appears that the increment in ovarian weight is roughly linear. The normal daily increment in ovarian weight in our strain of rats is shown by the dotted line in Figure 1, from which it can be calculated that the ovaries normally grow at an average rate of 0.05 mg. per hour.

TABLE 1. EFFECT OF INCREASING DOSAGE OF PMS ON RATE OF OVARIAN GROWTH BETWEEN 24 AND 48 HOURS AFTER A SINGLE INJECTION

Dosage in cc.	Average wts. 24 hrs.		Average wts. 48 hrs.		Rate of growth mg./hr.	
	No. animals	Av. ov. wt.	No. animals	Av. ov. wt.	Group av.	Grand av.
0.05	6	12.1±0.78*	8	14.8±1.01	0.10	
	8	11.8±1.03	9	12.8±1.24	0.04	0.05
	20	16.8±0.58	19	17.7±0.71	0.04	
0.10	12	13.6±0.63	13	16.4±0.53	0.12	
	6	11.0±0.76	6	18.4±1.14	0.28	0.14
	21	17.0±0.67	21	19.5±0.72	0.11	
0.15	16	18.7±0.38	18	23.9±0.94	0.30	0.30
0.25	5	13.1	6	26.1	0.51	
	10	18.3±1.03	9	25.9±2.53	0.32	0.50
	36	20.0±0.73	36	32.9±1.06	0.53	
0.40	5	18.7	6	29.1	0.41	0.64
	4	16.2	4	36.9	0.86	
0.50	22	19.4±1.00	22	40.5±1.60	0.88	
	4	17.3	7	36.6	0.77	0.80
	8	17.9±1.02	12	34.4±1.61	0.68	
0.60	22	23.5±0.91	22	44.4±1.86	0.87	0.87
0.75	32	18.9±0.62	32	44.7±1.67	1.07	
	3	13.6	3	28.8	0.63	1.00
	3	21.4	3	40.2	0.78	
1.00	7	22.0±2.02	8	41.1±2.20	0.91	
	10	22.2±1.14	10	56.2±2.57	1.35	1.11
	18	22.4±0.85	28	48.6±1.84	1.09	

* Standard deviations calculated by the formula $\sqrt{=\frac{Ed^2}{n(n-1)}}$.

In order to study in more detail the relationship between dosage and the rate of ovarian response to a single injection of PMS a large group of animals was injected with a single dose of serum. Half of the animals were killed 24 hours later, while the other half was killed 48 hours following the injection. To obtain the average hourly rate of ovarian growth at any given dosage the differences in ovarian weight at 48 hours and 24 hours were divided by 24, or by whatever time interval elapsed between the 2nd and 1st autopsy, since in some cases

autopsies could not be performed precisely at 24 and 48 hours. The average ovarian weights and the standard deviation of their means are given in Table I. The grand average was computed on the basis of the number of animals constituting each group average; i.e., a group average of 20 animals was given twice as much value as a group average of 10 animals.

The effect of increasing dosage of PMS on the rate of growth of

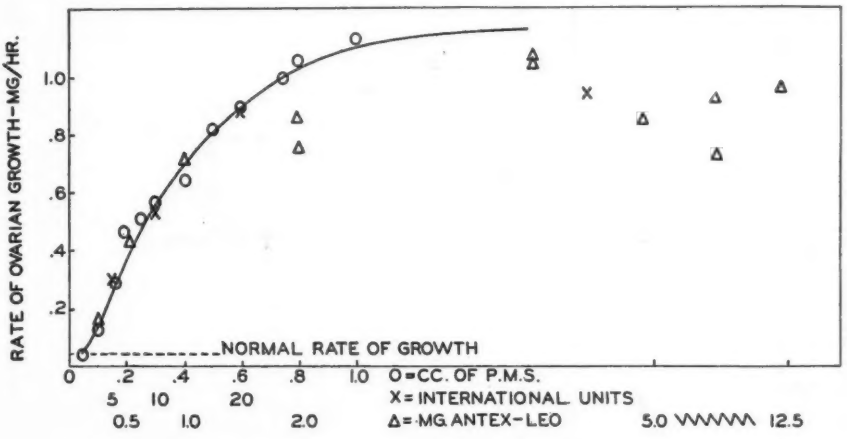


FIG. 2. The effect of a single injection of varying amounts of serum #29 in cc., of International Units of the International Standard (1 I.U. = 0.25 mg. International Standard preparation) and of Antex-Leo in mg. on the rate of ovarian response. Normal rate of growth in dotted line. The number of animals on each point of serum varied from 10 to 102, a total of 604 animals. A total of 117 animals was used for the International Standard, and a total of 199 animals for Antex-Leo.

the ovaries in milligrams per hour is shown in Figure 2. The points on this curve represent arithmetic averages (grand averages, Table I) of all data recorded for serum. The rate at which ovaries grow under the stimulation of PMS is expressed by a hyperbolic curve attaining a maximal rate quickly, and gradually declining to an asymtotic level at about 1.2 mg./hr. It is interesting that doses greater than 20 I.U. of the standard, and greater than 1 mg. of Antex-Leo produced less of a growth rate than equivalent amounts of serum. With smaller doses, however, the rates induced by these preparations fall on the smooth curve at the points for equivalent doses of serum.

1. Chorionic gonadotrophin

The same procedure outlined for PMS was used for PU. Groups of 21-day-old rats were given a single injection with various amounts (in micrograms) of Follutein. At 24 hourly intervals small groups

were sacrificed and the ovaries weighed. The results are shown in Figure 3. As with PMS, a single injection of PU causes a rapid growth of the ovaries between 24 and 48 hours after injection. Regression occurs after 48 hours, but in the greater doses, the active growth

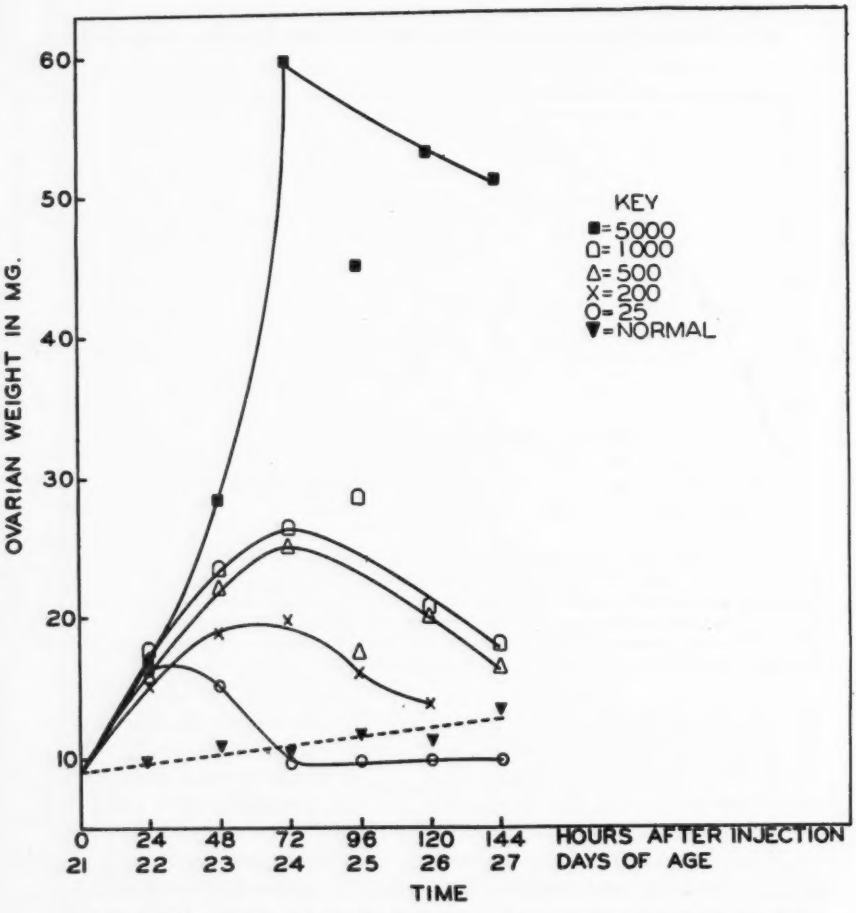


FIG. 3. The effect of a single injection of varying amounts of PU (Follutein) in micrograms on the response of the ovary at 24-hour intervals following injection. Normal daily ovarian weights are shown by the dotted line. Experimental points represent 5 animals each, a total of 150 animals.

phase may extend to 72 hours and beyond. Where there are several points along the active growth phase of these curves, the growth is roughly a linear function of time.

The effect of increasing dosage of PU on ovarian growth between

24 and 48 hours following a single injection is shown in Table 2. The same methods for computing the averages of growth as that described for PMS were used.

3. Comparison between pregnant mare serum and chorionic gonadotrophin

Although it may be deduced from Tables 1 and 2 that PMS and PU induce different rates of ovarian growth, it is obvious that these

TABLE 2. EFFECT OF INCREASING DOSAGE OF PU ON THE RATE OF OVARIAN GROWTH BETWEEN 24 AND 48 HOURS AFTER A SINGLE INJECTION

Dosage in micrograms	Average wts. 24 hrs.		Average wts. 48 hrs.		Rate of growth mg./hr.	
	No. animals	Av. ov. wt.	No. animals	Av. ov. wt.	Group av.	Grand av.
10	12	17.3±0.66	12	17.3±0.62	0.00	0
	5	15.6	5	15.4	0.00	
25	5	16.3	5	15.3	0.00	0
50	10	15.3±1.05	10	18.7±1.16	0.14	0.13
	4	14.8	5	17.3	0.12	
75	3	15.1	4	19.2	0.17	0.17
100	12	13.9±0.91	13	19.5±0.72	0.23	
	15	15.8±0.86	14	16.3±0.70	0.02	0.13
	10	17.4±1.10	10	20.5±1.20	0.13	
200	5	15.0	5	18.9	0.16	
	14	13.8±0.61	15	18.9±0.82	0.22	0.22
	10	16.1±0.93	10	22.4±1.29	0.26	
	13	15.2±0.51	14	20.0±0.94	0.21	
500	11	18.5±1.23	11	21.9±0.50	0.14	
	12	14.6±0.70	11	21.3±1.46	0.28	0.25
	15	18.2±0.87	15	26.8±1.18	0.32	
	4	16.0	5	22.0	0.25	
1000	7	16.5±0.64	8	22.2±1.48	0.23	
	10	17.3±1.68	10	21.9±2.52	0.19	
	8	16.5±0.98	8	25.2±2.22	0.36	0.23
	5	17.3±1.16	5	23.3±1.65	0.25	
	8	17.1±1.09	8	20.5±1.11	0.14	
5000	10	15.9±0.57	5	28.4±3.38	0.52	0.52

hormones can be compared only on some mutual basis of activity. A comparative unit in terms of amounts of each hormone necessary to induce a given weight of the ovaries was used, namely, that amount of substance which when given subcutaneously to 21-day-old rats in quartly doses, once daily for 4 days induces a doubling of ovarian weight (24 mg.) on the 5th day. This unit was carefully determined by repeated tests along with a simultaneous similar assay of the International Standard preparation of each hormone.

For pregnant mare serum (#29), we have found that one gonadotrophic unit as described above is contained in 0.2 cc. serum, and is equivalent to 6.6 International units of PMS or 1650 micrograms of the International Standard preparation. For PU, one unit is 30 micrograms of Follutein, equivalent to 10 I.U. or 1 mg. of International Standard preparation. Therefore 6.6 I.U. of PMS and 10 I.U. of PU each contain one gonadotrophic unit. By transposing these values in terms of rat units, the data for PMS and PU were replotted in Figure 4, showing the effect of equivalent rat units of PMS and PU on

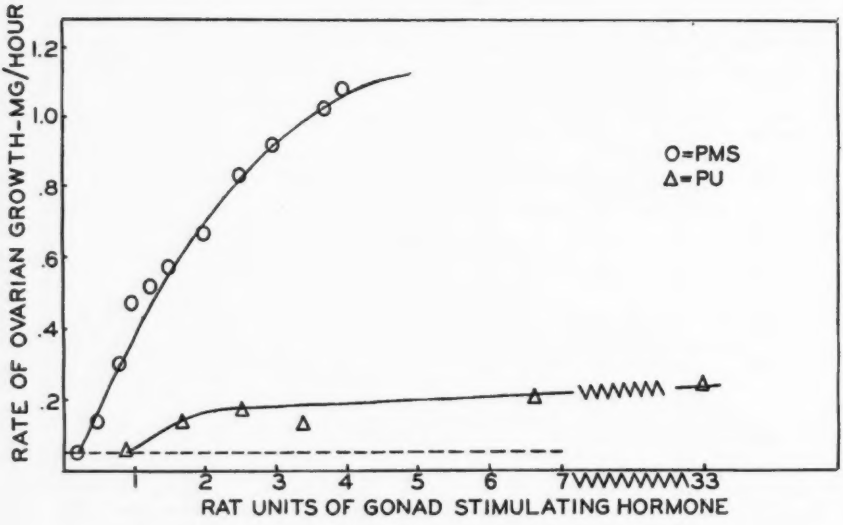


FIG. 4. The effect of physiologically equivalent doses of PU and PMS (in terms of rat units) on the rate of ovarian growth between 24 and 48 hours following a single injection.

the rate of ovarian growth. The greater effectiveness of PMS is at once apparent. The entire range of the curve is covered between ¼ and 4 rat units of PMS. For PU, there is no effect on the rate of growth until one rat unit is given, and the maximal rate induced by approximately 170 rat units of PU is only 0.5 mg./hr., less than half the asymptotic level for PMS, and equal to a little more than 1 R.U. of PMS.

Among a few significant considerations, which will be discussed shortly, resulting from the differential characteristic effects of these hormones on ovarian growth, one can be brought out at this point. Given an unknown preparation which is either PMS or PU, the identification can be easily established by injecting 5 rat units and determining the rate of response. If the rate is 0.25 mg./hr. or less the substance is PU. If it is four or more times greater, it is PMS. This

requires no special procedures such as the use of hypophysectomized animals or the more laborious method of augmentation by FSH.

DISCUSSION

The purpose of this paper is to present a new method of studying gonadotrophic hormones in terms of the rate at which they are capable of inducing ovarian growth. The characteristics of two such agents are presented and shown to be vastly different.

There are undoubtedly several reasons why PU and PMS exert such different effects on ovarian growth. These substances are chemically quite different. Biologically, PMS is predominantly follicle stimulating, PU mainly luteinizing. PMS is active in hypophysectomized female rats; PU is not. However, in the first 48 hours of response in a normal immature rat these biological differences assume a lesser significance. Finally it is possible that the differences may be partly explainable on a pharmacodynamic basis since PMS is regarded as not being passed through the kidney while PU is readily filtered. However, it is manifestly impossible to approximate the effects of PMS by giving virtual saturation doses of PU. Such factors as elimination and destruction, therefore, do not seem important. It appears likely, then, that each gonadotrophic hormone will be as well defined by its characteristic biological effect on rates of ovarian growth as by any other property, and that probably no two substances will induce the same kinetic effect on the ovaries.

Several minor aspects of the action of these gonadotrophic agents can now be clarified. There has been some confusion concerning the efficacy of single versus divided doses, and the time of maximal activity. These difficulties arise because time and dosage have not been considered simultaneously. For example, the maximal response (i.e., greatest weight of ovaries produced) of PMS has usually been considered to be 96 hours following a single injection. This is certainly true of large doses, but reference to Figure 1 shows that the time of maximal weight for both large and small doses is directly proportional to dosage. The question of single versus divided doses can be evaluated from several approaches. Leathem has stated that PMS (large doses) give greater ovaries 120 hours after a single subcutaneous injection than the same dose divided in quarterly portions and given daily for 4 days.

For small doses of PMS we have shown that 0.15 cc. PMS will produce 24 mg. ovaries in 48 hours whereas 0.2 cc. is required to produce the same response in 108 hours when given in quarterly doses of 0.05 cc. per day. Thus it would seem that PMS given in a single injection is more effective than when given in multiple injections. However, if the response is compared at 108 hours, it would perhaps take two times as much hormone given as a single dose to produce 24 mg. ovaries, since 108 hours represent a phase of regression for

most doses. Thus, for smaller doses at least, it can be concluded that multiple injections are more effective than a single injection. Such opposing conclusions can be drawn when only one of the two variables, time and dosage, is considered. The common denominator is the type curve shown in Figures 1 and 2, where each curve represents the history of the dosage injected. When multiple injections are given, then a series of such curves would result. Daily small doses such as 0.05 cc., each of which by itself induces no reaction in 24–48 hours, may become cumulative so that by the 4th day a sufficient blood level is built up to induce a response.

The use of rate curves as method of assay for these substances merits little discussion, for the establishment of the International Standard for both these substances makes all other units superfluous. Furthermore, for PU the rate effect is too limited. For PMS, however, the rate curve can be used as a 48-hour assay. Its only possible advantage over the method of Cole and Erway is that the variation in determining rate is less than the variation encountered in using only the 48-hour reading, for in most cases, the 48-hour reading is high when the 24-hour reading is high, and low when the first reading is low. As a point of interest, we can give the following equivalents based on a growth rate of 0.3 mg./hr. for PMS, a point about halfway along the steepest part of the curve. At this rate, 5 I.U. of the International Standard = 0.15 cc. PMS (#29) = 3.75 rat units (Cole) = 0.75 rat unit (described in this paper, 4-day test). Cole, Pencharz and Gross (1940) state their rat unit equal to about 2 I.U. and later, (Cole and Erway) to about 1 unit. We calculate 1 rat unit (Cole, corpora lutea method) equal to 1.3 I.U., a fairly good agreement.

SUMMARY

The effect of pregnant mare serum and chorionic gonadotrophin on the rate of ovarian growth in 21-day-old rats has been determined by giving 1 dose and weighing ovaries at 24 and 48 hours following a single injection. It has been found that physiologically equal doses of PMS and PU affect the rate of growth quite differently, each in a characteristic fashion which cannot be duplicated by any adjustment of dosage of one or the other. The significance of these characteristic rate curves for PMS and PU has been discussed with the viewpoint of their use in the determination of unknown preparations, qualitatively and quantitatively.

ACKNOWLEDGEMENT

We are indebted to E. R. Squibb & Sons for a generous supply of Follutein used in this study.

REFERENCES

CATCHPOLE, H. R., AND W. R. LYONS: *Am. J. Anat.* 55: 167. 1934.
COLE, H. H., H. R. GUILBERT AND H. GROSS: *Am. J. Physiol.* 102: 227. 1932.
COLE, H. H.: *Am. J. Anat.* 59: 299. 1936.
COLE, H. H., AND J. ERWAY: *Endocrinology* 29: 514. 1941.
COLE, H. H., R. I. PENCHARZ AND H. GROSS: *Endocrinology* 27: 548. 1940.
EVANS, H. M., K. MEYER AND M. E. SIMPSON: *Mem. Univ. Calif.* 11: 257. 1933.
HAMBURGER, C., AND K. PEDERSEN-BJERGAAD: *Quart. J. Pharm. & Pharmacol.* 11: 186. 1938.
LEATHEM, J. H.: *Endocrinology* 28: 615. 1941.

TOLERANCE OF NORMAL, OF THYROIDECTOMIZED, AND OF THIOUREA OR THIOURACIL TREATED DOGS TO ORAL DESICCATED THYROID AND TO INTRAVENOUS THYROXINE[1]

DANOWSKI, T. S., MAN, E. B. AND WINKLER, A. W.
From the Departments of Medicine and of Psychiatry, Yale University School of Medicine

HUMAN PATIENTS with myxedema can not take oral desiccated thyroid in amounts greater than about 0.2 gram daily without developing a supranormal level of precipitable iodine in serum along with symptoms and signs of hyperthyroidism (Winkler, Criscuolo and Lavietes, 1943; Winkler, Riggs and Man, 1945). Tolerance never develops. Euthyroid subjects, on the other hand, can often tolerate indefinitely much larger amounts of desiccated thyroid, 0.3 to 0.9 gram daily, before metabolic rate and precipitable iodine of serum increase (Riggs, Man and Winkler, 1945; Peters, Danowski and Winkler). The ingested hormone is apparently not stored by euthyroid subjects, since there is no evidence of its eventual release when thyroid medication is discontinued. A difference in response to intravenous thyrosine between myxedematous patients and euthyroid subjects has also been noted (Means, 1937; Winkler, Lavietes, Robbins and Man, 1943). As far as is known the myxedematous subject adequately treated with thyroid medication differs from the euthyroid subject only in having no thyroid tissue (Means, 1937). It has been suggested, therefore, that in man ingested thyroid hormone within certain limits may be promptly inactivated by the thyroid gland itself, and that other tissues possess this capacity to a limited extent only.

Thiourea and thiouracil produce functional hypothyroidism in both human subjects and in animals, yet do not destroy the thyroid gland (Astwood, 1943. The present studies were originally undertaken to determine the reaction of subjects rendered hypothyroid in this manner to thyroid and thyroxine medication. The problem has practical implications, since recently desiccated thyroid has been employed clinically to correct the myxedema induced by thiouracil and thiourea (Palmer, 1945; Danowski, Winkler, and Man, 1945). The responses to oral thyroid and to intravenous thyroxine of dogs treated with one of these drugs were compared to those of normal

Received for publication February 19, 1946.
[1] Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

and of thyroidectomized animals. The results indicate that the dog, much more readily than the human subject, inactivates large amounts of thyroid hormone in tissues other than the thyroid gland. Furthermore, in the dog the response to thyroxine is apparently determined by factors other than the status of the thyroid gland. Other important differences between dog and man in the physiological role of the thyroid were also discovered. The experiments are therefore reported here primarily because of their significance in comparative physiology.

MATERIALS AND METHODS

Male and female dogs weighing 6 to 16 kilograms were used. Nine animals received desiccated thyroid, U.S.P., orally in doses of 0.77 gram daily for periods of 5 to 16 weeks. Four animals were completely thyroidectomized. One or more parathyroid glands were preserved. Three other animals received a preliminary course of either thiourea or thiouracil,[2] orally in 2.0 gram doses daily for periods of 28 to 40 weeks. This dosage was continued throughout the experimental period in all 3 of these animals, and during the recovery period in 2 of the animals. Two other dogs with intact and untreated thyroid glands served as controls.

In another series of 15 experiments thyroxine was given intravenously in doses of 2.0 milligrams daily for 13 to 37 days to dogs from 3 comparable groups of animals. The thyroxine was injected as a 40 milligrams per cent solution in 0.02 N NaOH. Two of the 4 thyroidectomized dogs in this group had the parathyroid glands removed as well. Normal concentrations of serum calcium were maintained in these by the administration of calcium lactate and dihydrotachysterol. The 4 animals in the thiouracilized group received a preliminary course of this drug 6 to 52 weeks in length.

The concentration of precipitable iodine in serum (Riggs and Man, 1940; Man, Smirnow, Gildea and Peters, 1942) was followed in each animal before, during, and, when possible, after treatment. In all animals body weight and in many others the pulse rate, basal oxygen consumption, and the food intake were measured as well. The rate of oxygen utilization was obtained after preliminary training by means of a Benedict-Roth machine fitted with a cone and a rubber diaphragm coated with vaseline.

Autopsy confirmation of the status of the thyroid gland was obtained in each animal. The control animals had normal glands. One of the thyroidectomized animals, Dog 54, had regenerated a nodule of thyroid tissue 3 mm. in diameter when examined 18 months later. No thyroid tissue could be found, either aberrant or in the thyroid bed, in the other 3 animals. The thiourea or thiouracil treated dogs developed characteristic hyperplasia of the thyroid gland.

RESULTS

Concentration of precipitable iodine in serum. The serum precipitable iodine values in untreated dogs with intact thyroid glands

[2] All 3 dogs were started on thiourea. Dog 23 was continued on thiourea throughout the experiment. Dogs 47 and 50 were shifted to thiouracil 16 and 9 weeks respectively before the administration of thyroid. The thiouracil ("Deracil") was supplied by the Lederle Laboratories through the courtesy of Dr. Stanton M. Hardy.

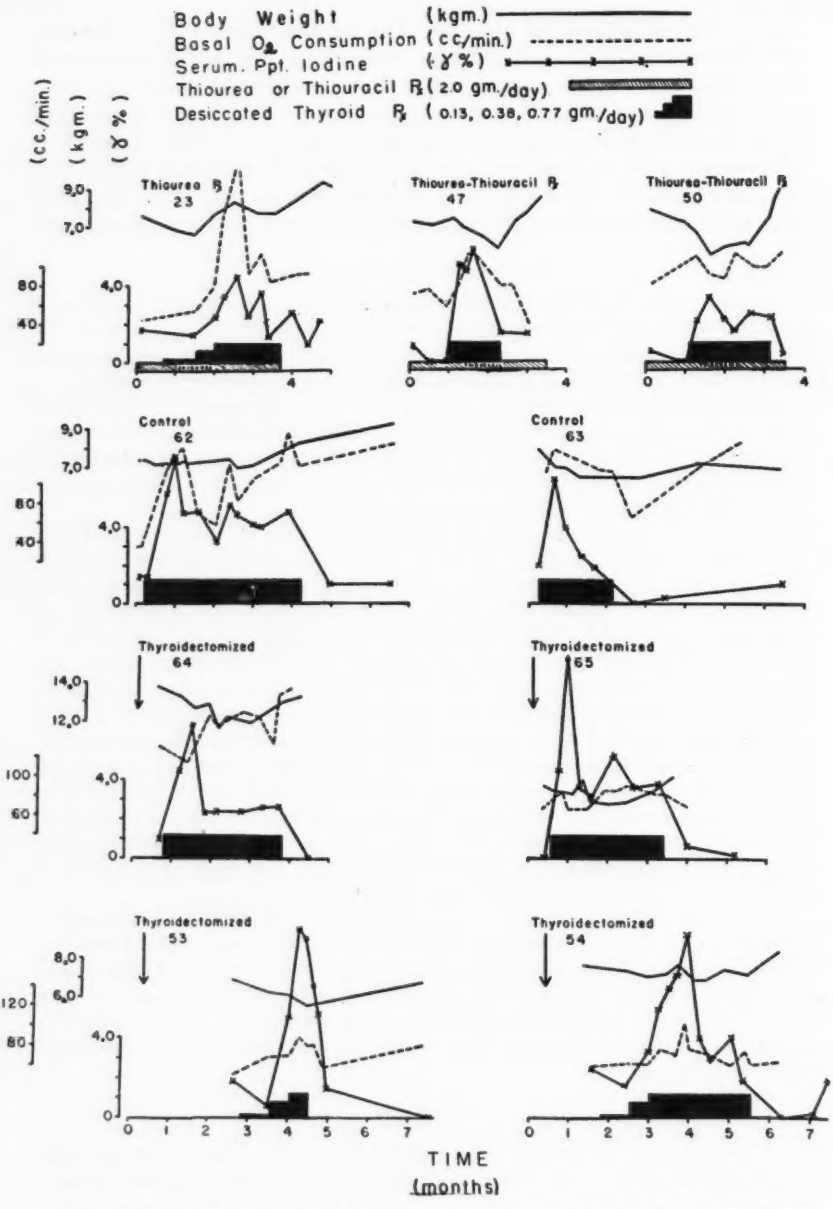


FIG. 1. Effect of desiccated thyroid on the serum precipitable iodine, the basal oxygen consumption, and the body weight of (a) thyroidectomized, (b) thiourea or thiouracil treated, and (c) control dogs.

ranged from zero to 2.4 gamma[3] per cent (Figures 1 and 2). These concentrations are much lower than the 3.0 to 8.0 gamma per cent range observed in euthyroid human subjects (Riggs and Man, 1940).

Response to exogenous thyroid. Exogenous thyroid hormone is destroyed or inactivated in the dog at a rate which is apparently uninfluenced by the presence or absence of the thyroid gland, or by its functional state. Ingestion of small doses of thyroid, 0.13 gram daily, by Dogs 23, 53, and 54 produced no significant rise in the serum precipitable iodine or in metabolic rate. Ingestion of 0.38 gram of thyroid daily by the same 3 dogs was followed by a slight rise in serum precipitable iodine (2.4, 5.0, and 3.3 gamma per cent, respectively), with only slight, if any, increase in metabolic rate (Figure 1). Ingestion of 0.77 gram of thyroid daily by control, thyroidectomized, and thiourea or thiouracil treated dogs was regularly attended by an increase in the concentration of serum precipitable iodine up to as much as 9.3 gamma per cent within 7 to 14 days of therapy (Figure 1). This was usually, but not invariably, correlated with a decrease in body weight, and an increase in oxygen consumption. The peak of the latter coincided with the maximum recorded concentration of serum precipitable iodine. In some animals, however, the peak became manifest later, at a time when the iodine concentration was falling.

Continuation of thyroid administration was followed in the third to fourth week by a partial or complete return of the serum precipitable iodine to the normal range. Thereafter no further change occurred. A decrease in oxygen consumption usually accompanied this drop. With this adjustment the animals regained weight.

Cessation of thyroid therapy resulted in a drop in serum iodine to zero in all of the thyroidectomized animals, and in one of the two control dogs. Subsequently, the serum precipitable iodine returned to the pre-treatment level in this animal (Dog. 63).

Response to thyroxine. No clear difference was demonstrable among the three groups of animals in their reaction to intravenous thyroxine. In Figure 2 it is readily evident that there was no response of the serum precipitable iodine characteristic either of thyroidectomized or of the thiouracil treated dogs which would serve to differentiate either group from the control group. Twenty-four hours after the first thyroxine injection the serum precipitable iodine was found to be sharply elevated. Continuation of the thyroxine was followed by a further increase in the next 2 weeks. Thereafter the concentration of serum precipitable iodine either rose less sharply, remained unaltered or, in 3 experiments, dropped slightly despite continuation of thyroxine. These responses were unrelated to the initial body weight.

[3] The value "zero" throughout the paper refers to concentrations not significantly different from the blank. It does not necessarily indicate, however, that precipitable iodine was totally absent from serum.

The rise in serum precipitable iodine was associated with an increased oxygen consumption in 8 of the 11 animals in which this measurement was made, and with an increased pulse rate in 10 out of 14 animals. The body weight decreased in all but two experiments. Two

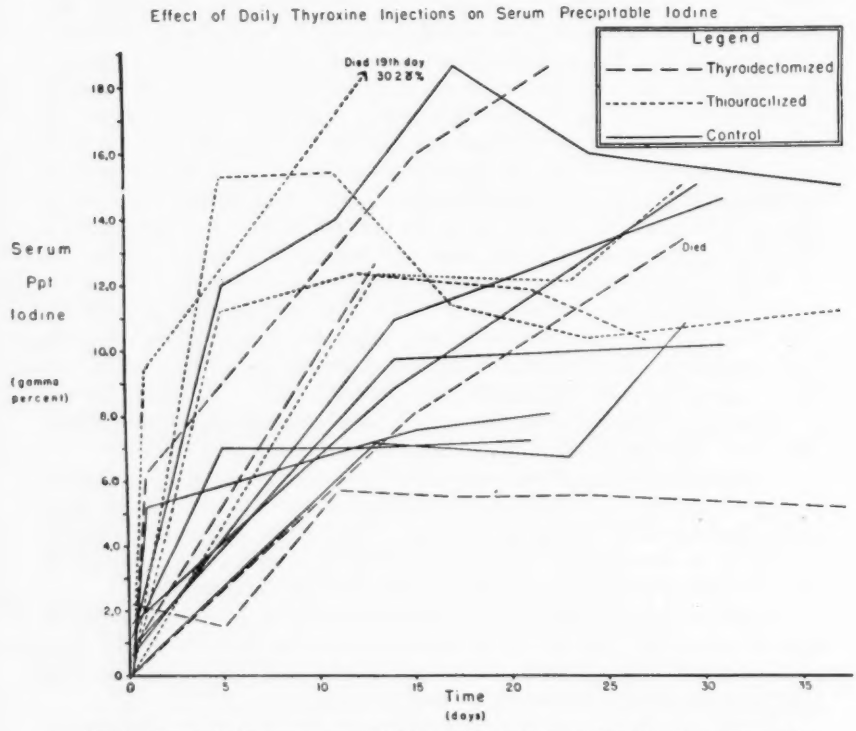


FIG. 2. Increase in serum precipitable iodine produced by daily intravenous injection of 2.0 milligrams of thyroxine in thyroidectomized, thiouracil treated, and control dogs.

of the animals which had lost a great deal of weight died during the course of thyroxine medication.

Clinical and biochemical changes in totally thyroidectomized animals. No consistent change in the appearance or behavior of two completely thyroidectomized dogs developed during a year or so of observation, although each was maintained without replacement therapy. During these periods without substitution therapy serum precipitable iodine was not significantly different from zero, and the basal oxygen consumption declined in both animals. Sometimes they tended to lose weight due to loss of appetite, but this difficulty was overcome by special attention to feeding.

DISCUSSION

The dog differs from man in several aspects of thyroid physiology. (1) The level of circulating hormone, as measured by the serum precipitable iodine, is always much lower than it is in human subjects, and may normally be undetectable in the serum. (2) The dog, in proportion to the body weight, has a much greater capacity than does man to inactivate or destroy exogenous thyroid hormone. With desiccated thyroid in 0.77 gram daily doses a stimulation of the metabolism occurred with an increased consumption of oxygen and a loss of body weight despite a well-maintained intake of food. With continued ingestion of thyroid this initial maximal response was followed in most animals by a return of serum iodine, oxygen consumption, and body weight toward the pre-treatment levels. In comparable per kilogram doses the 0.77 gram administered daily to a 7 kilogram dog would represent a daily intake of 5 to 8 grams in human subjects, an amount approximately forty times the usual therapeutic dose in myxedema. (3) The totally thyroidectomized dog possesses this ability to inactivate hormone to about the same degree as the normal dog. This ability appears to reside primarily, if not entirely, in the extrathyroid tissues. The possibility exists that the thyroid gland possesses the capacity to destroy excess hormone. However, the identity of the responses to exogenous thyroid obtained with thiourea and thiouracil treated, thyroidectomized and non-thyroidectomized animals indicates that this mechanism is quantitatively insignificant, if it exists at all, in dogs. The drop in serum precipitable iodine levels to zero in two weeks following cessation of thyroid therapy in a normal dog indicates that during the administration of exogenous thyroid the elaboration of endogenous hormone is suppressed. This has already been observed in human subjects (Riggs, Man and Winkler, 1945). This suggests that the ability to tolerate large amounts of thyroid is mediated not only by increased destruction or utilization in the extrathyroid tissues, but also by decreased secretion of endogenous hormone. The latter mechanism is probably much less significant quantitatively. (4) Total thyroidectomy or thiourea and thiouracil do not produce any clinical or chemical syndrome in the dog corresponding to the myxedema which develops in man. Although serum iodine fell to zero, this situation is not necessarily abnormal in the dog. Oxygen consumption fell irregularly and comparatively slightly. Chaikoff and his associates (1941) and Thompson and Long (1941) could only occasionally demonstrate a persistent increase in the serum cholesterol after total thyroidectomy. These slight and sporadic changes which characterize the athyroid dog contrast sharply with the clinical and chemical syndrome of hypothyroidism which develops within a few weeks in human subjects following thyroidectomy or withdrawal of thyroid medication in patients with myxedema.

These differences between dog and man are probably more quanti-

tative than qualitative in character. The less marked disturbance produced by total thyroidectomy in dog compared with man indicates that the dog's tissues are much less dependent upon hormone received from the thyroid gland than are those of man. Other species, such as the monkey, behave much as does the dog (Fleischman, Shumacker and Straus, 1943; Jailer, Sperry, Engle and Smelser, 1944). The differences between dog and man seem, however, to be one of degree rather than kind, since there is abundant evidence that the metabolism of the dog is affected by the thyroid hormone. Despite a normally low concentration of serum precipitable iodine, a further decrease could be produced by thyroidectomy or by thiourea or thiouracil, associated with a fall in the basal oxygen consumption (not shown in Figure 1). The hypometabolic state produced in this way could be reversed promptly either by withdrawal of the thyroid depressant drug or by feeding thyroid. This certainly indicates that thyroid hormone plays a role in the metabolic processes of dog tissues, inasmuch as thiourea and thiouracil exert their effect only by preventing formation of the thyroid hormone. The low level of circulating hormone may be the result of either a decreased production by the thyroid gland, or of an increased utilization of the hormone by the tissues, or of both. The second explanation is supported by the extraordinary ability of the tissues to inactivate exogenous hormone, the former by the very gradual decline of the serum precipitable iodine following thiouracil treatment. For the present the question must be left undecided. Evidence has recently been advanced suggesting extrathyroidal formation of thyroid hormone (Morton, Chaikoff and Rosenfeld, 1944). It is impossible, on the basis of these experiments of ours, to determine whether the relative independence of the tissues of the dog from the control of the thyroid gland is due to real insensitivity or to some ability to manufacture their necessary hormone outside the thyroid.

Dempsey and Astwood (1943) have suggested that the minimum daily dosage of thyroid hormone necessary to prevent thyroid hyperplasia in rats under the influence of thiouracil is equivalent to the daily output of hormone by the normal animal. On the basis of this assumption they as well as other workers have sought to compare experimentally the potency of various thyroid and thyroxine preparations. Underlying this method of assay is the assumption that ingested thyroid and injected thyroxine are utilized by the peripheral tissues as effectively as the naturally produced hormone. The experiments reported in this paper indicate that, under certain circumstances, large amounts of exogenous thyroid hormone can be destroyed without a demonstrable effect on the metabolism. This observation suggests the possibility that, in bioassays by the method of Dempsey and Astwood, partial inactivation of the administered hormone may occur. This would of course lead to an overestimation of the daily rate of production of the natural hormone. The inconsistency

between the results obtained by various workers (Dempsey and Astwood, 1943; Reinecke, Mixner and Turner, 1945; Reinecke and Turner, 1945) could possibly be explained in terms of variation in the extent of inactivation of exogenous hormone. No critical experiments which support or refute this suggestion are, however, at present available.

SUMMARY AND CONCLUSIONS

The dog and man differ in their thyroid physiology in certain respects. The level of circulating hormone, as measured by the serum precipitable iodine, is always much lower than it is in human subjects, and the concentration may normally be too low to detect. The dog, in proportion to the body weight, has a much greater capacity than does man to inactivate or destroy exogenous oral thyroid or intravenous thyroxine. In contrast to human subjects this ability appears to reside primarily, if not entirely, in the extrathyroid tissues. Total thyroidectomy or chemical inhibition of formation of hormone in the thyroid gland by thiourea or thiouracil does not produce in the dog any syndrome comparable to human myxedema. These differences indicate that the metabolic processes of the dog are less dependent than are those of man on the hormone produced by the thyroid gland.

ACKNOWLEDGMENT

We are indebted to Dr. Samuel Harvey and to Dr. John Lockwood for the use of the laboratories of the Department of Surgery.

REFERENCES

ASTWOOD, E. B.: *J.A.M.A.* 122: 78. 1943.
CHAIKOFF, I. C., C. ENTENMAN, G. W. CHANGUS AND F. L. REICHERT: *Endocrinology* 28: 797. 1941.
DANOWSKI, T. S., E. B. MAN AND A. W. WINKLER: *Amer. J. Med. Sciences* 210: 777. 1945.
DEMPSEY, E. W., AND E. B. ASTWOOD: *Endocrinology* 32: 509. 1943.
FLEISCHMAN, W., H. B. SHUMACKER, JR. AND W. L. STRAUS, JR.: *Endocrinology* 32: 238. 1943.
JAILER, J. W., W. M. SPERRY, E. T. ENGLE AND G. SMELSER: *Endocrinology* 35: 27. 1944.
MAN, E. B., A. E. SMIRNOW, E. F. GILDEA AND J. P. PETERS: *J. Clinical Investigation* 21: 773. 1942.
MEANS, J. H.: The Thyroid and Its Diseases. J. B. Lippincott Company, Philadelphia, 1937.
MORTON, M. E., I. L. CHAIKOFF AND S. ROSENFELD: *J. Biol. Chem.* 154: 381. 1944.
PALMER, M. V.: *Ann. Int. Med.* 22: 335. 1945.
PETERS, J. P., T. S. DANOWSKI AND A. W. WINKLER: Unpublished data.
REINECKE, E. P., J. P. MIXNER AND C. W. TURNER: *Endocrinology* 36: 64. 1945.
REINECKE, E. P., AND C. W. TURNER: *Endocrinology* 36: 200. 1945.
RIGGS, D. S., AND E. B. MAN: *J. Biol. Chem.* 134: 193. 1940.
RIGGS, D. S., E. B. MAN AND A. W. WINKLER: *J. Clin. Investigation* 24: 722. 1945.
THOMPSON, K. W., AND C. N. H. LONG: *Endocrinology* 28: 715. 1941.
WINKLER, A. W., J. CRISCUOLO AND P. H. LAVIETES: *J. Clin. Investigation* 22: 531. 1943.
WINKLER, A. W., P. H. LAVIETES, C. L. ROBBINS AND E. B. MAN: *J. Clin. Investigation* 22: 535. 1943.
WINKLER, A. W., D. S. RIGGS AND E. B. MAN: *J. Clin. Investigation* 24: 732. 1945.

THE INFLUENCE OF ESTROGENS ON THE CRYSTAL STRUCTURE OF BONE AS REVEALED BY X-RAY DIFFRACTION STUDIES ON THE FEMORA OF MICE[1]

C. I. REED AND B. P. REED

From the Department of Physiology, University of Illinois, Chicago Colleges

W. U. GARDNER

From the Department of Anatomy, Yale University

THE INFLUENCE of estrogenic substances on the gross structure and composition of bone has been the subject of numerous studies, anatomical, histological and chemical. An extensive review of the literature has been made (Gardner & Pfeiffer, 1943).

The possibilities of correlating the minute physical structure of bone with the physiological changes induced by estrogens became apparent after extensive examination of rats' tibiae by x-ray diffraction technic. In recent publications, Reed and Reed (1942, 1945) have confirmed the close similarity of powdered apatite and powdered bone previously reported by several workers, and have agreed with Klement (1938) that the formula frequently used for apatite $Ca_{10}(PO_4)_6\begin{bmatrix}CO\\Cl_2\\OH\\Fl\end{bmatrix}$ applies as well to bone salts. Also, it was shown that disorientation may take place very rapidly, within 48 hours, and be restored again after several weeks. While there is no very close correlation between radiological evidence of healing of rickets and complete reorientation, in general it appears that this change does not occur until the rachitic state is completely healed. It has been shown also that reorientation is not closely correlated with restoration of lateral breaking strength in rachitic bones.

There appears to exist good evidence that vitamin D, when added to a rachitogenic diet in adequate amounts is capable alone, of effecting reorientation. While the synthesis of the apatite molecule, which is not formed in the body except in bone, may depend on other factors than vitamin D, its arrangement in the bone may depend on the vitamin to a considerable extent.

Received for publication February 21, 1946.

[1] This investigation has been supported by grants from The Fluid Research Fund of Yale University and The Jane Coffin Childs Fund for Medical Research.

It has been assumed too commonly that the mineral deposit occurring in bones of animals treated with estrogens represents ossification of the type found in normal bone. The present study was undertaken in an effort to apply the technic already developed for identifying crystalline patterns to the identification of the inorganic matrix of the deposits in femora of estrogen-treated mice.

MATERIALS AND METHODS

The bones were taken from mice prepared in New Haven and were sent to Chicago for diffraction studies with no clue as to the previous history. The data obtained from the specimens and diffractograms were then compared with the histories. The animals were both male and female mice of the C_3H strain 40 to 60 days old when pellets of estrone weighing 2 to 3 mg. were

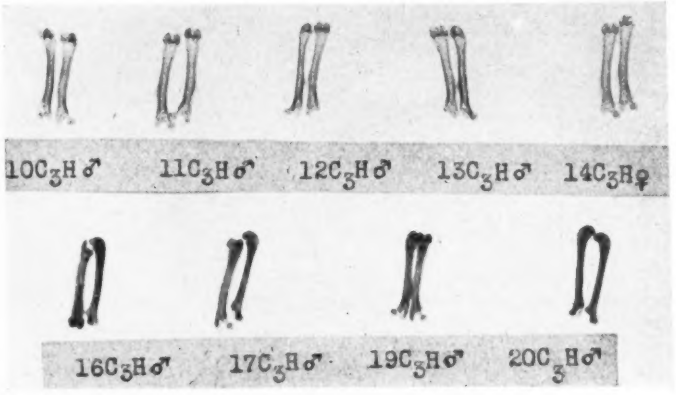


FIG. 1. A print from a roentgen photograph of the femurs of five control and 4 estrogen-treated mice. All femurs were photographed at the same time. Note the increased density in the femurs of the estrogen-treated mice.

implanted subcutaneously. Although the actual dosage cannot be determined accurately because of the variable rates of absorption this method of administration assures absorption of amounts of hormone in excess of minimal effective doses to incite maximal osseous changes. There were 11 males and 8 females in the control group and 9 males and 3 females in the treated group. The treated animals were kept 84 to 89 days after implantation. The greater part of the pellets were present at death. The controls were killed from 84 to 97 days after the experiment started.

Both femora were removed, partially cleaned and dried and then the remaining soft tissues were removed. Radiograms were taken (Fig. 1) to reveal the extent of mineralization of the medullary canals although the extent of medullary ossification could usually be determined satisfactorily by merely inspecting the broken ends of the shafts under a hand lens. When medullary deposits were present two specimens were prepared for diffractograms, one by grinding through the shaft so as to expose only original cortex, the other by grinding off the cortex and saving the medullary de-

posits. In some bones, the division between the two (cortical, medullary) layers could be detected visually, but in others there was sufficient fusion so that it was difficult to be sure of a complete separation.

In Fig. 2 is shown a mesial sagittal surface of one of the femora of a treated mouse which emphasizes particularly the disorganized state of such bones.

OBSERVATIONS

Figure 3 shows a diaschema of apatite and bone powder reproduced from an earlier paper that will serve as a reference pattern diffractogram in localizing all descriptions in this paper. Figure 4

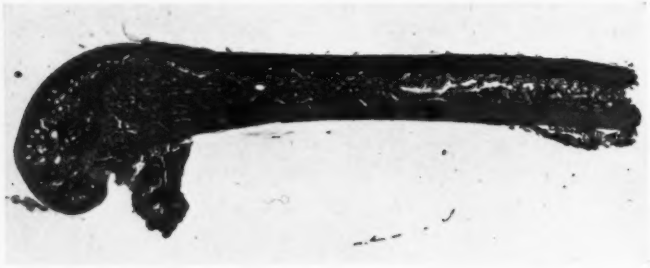


FIG. 2. A section through the distal end and diaphysis of the femur of a mouse of the C_3H strain which had received a pellet of estrone 71 days prior to the removal of this specimen. The epiphysial line is still evident, the circumferential cortical bone is compact beyond the center of the femur. The bone at the distal end of the femur (left) and in the medulla is irregularly arranged.

shows a diffractogram from the diaphysis of a femur of a normal mouse. It is, however, difficult to bring out in a published reproduction the fine details clearly visible in the original negative film. There is preferred orientation in five of the rings—Nos. 5, 8, 10, 12 and 13—manifested by arcing or fibering. The longitudinal axis of the bone is at right angle to the arcs in No. 5. When such a bone is powdered, the preferred orientation disappears and the rings become continuously of uniform thickness, as in Figure 3. Disorientation in rickets, or after parathyroid hormone, is manifested in the same way. In a diffractogram of powdered bone obtained from a healthy rat, the crystal spacings in Angström units and the planar indices were found as shown in Table 1. The bones of untreated mice give patterns comparable to those of the rat.

So far as could be determined by this method, there was no difference in the responses of the bones of males and females to estrone. Four pregnant females in the control group showed no disorientation. Table 2 shows the data on all animals.

In 4 of the treated mice the medullary canals were still visible although narrowed; in all others they were filled with osseous tissue. In 8 animals the diffractograms showed complete disorientation in all

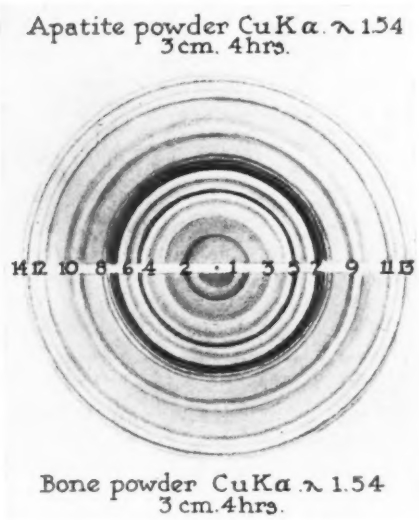


FIG. 3. Diffractogram of bone powder and apatite.

rings; in 2 there was marked disorientation, while in the remaining 2 this change was less pronounced although still definite. Thus, it can be said that there was disorientation in the cortices of all femora. The material deposited in the medullary canal was always completely unoriented.

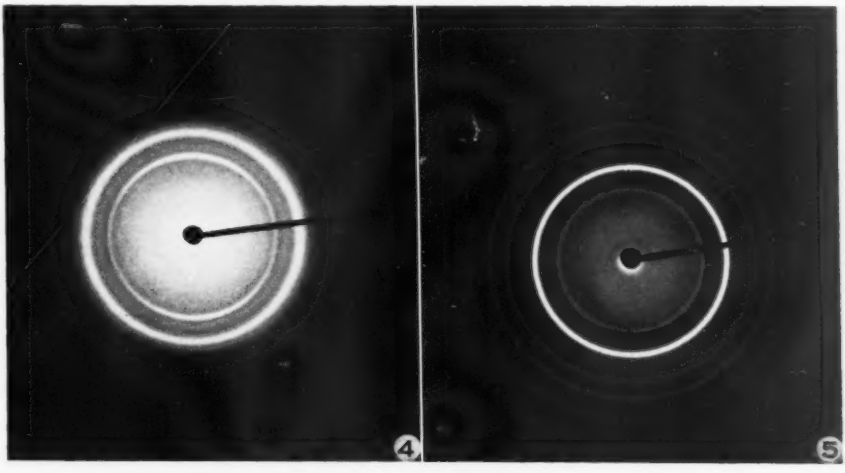


FIG. 4. Diffractogram from the diaphysis of the femur of a normal mouse.
FIG. 5. Diffractogram of powdered egg shell.

TABLE 1

Ring No.	$\frac{d}{A}$	Indices	Orientation
3	4.90	$11\bar{2}0$ $20\bar{2}0$	none
4	3.85	$11\bar{2}1$	none
5	3.50	$20\bar{2}1$	marked
6	3.22	$10\bar{1}2$	none
7	2.84	$21\bar{3}1$	none
8	2.67	$30\bar{3}0$	marked
9	2.30	$21\bar{3}2$	none
10	2.00	$11\bar{2}3$	marked
11	1.85	$32\bar{5}0$	none
12	1.63	$32\bar{5}2$	marked
13	1.58	$31\bar{4}3$	marked

Nos. 1 and 2 are referable to the large particles of organic material.

In the control group the medullary canals were open and there was complete orientation in all with the exception of the femora of 2 animals, where a very mild degree of disorientation was detected.

TABLE 2

No.	Sex	Days observed	Disorientation	Medullary canal	$CaCO_3$ detected
			Estrogen Treated Mice		
1	M	84	Mild	Narrowed	0
2	M	84	Moderate	Narrowed	0
5	M	84	Complete	Closed	+
6	M	84	Complete	Closed	+
7	M	84	Complete	Closed	?
16	M	84	Marked	Almost closed	?
17	F	89	Complete	Closed	+
19	F	89	Complete	Closed	+
20	M	89	Complete	Closed	+
21	M	89	Complete	Closed	+
32	F	84	Marked	Almost closed	+
34	M	84	Complete	Closed	+
			Controls		
9	M	84	0	Open	0
10	M	84	0	Open	0
11	M	84	0	Open	0
12	M	84	0	Open	0
13	M	84	0	Open	0
14	M	84	0	Open	0
22	M	90	0	Opeu	0
23	M	90	0	Open	0
24	F	90	0	Open	0
25	F	90	0	Open	0
26	F	90	0	Open	0
27	M	90	0	Open	0
28	M	90	0	Open	0
35	F	97	0	Open	0
36	F	97	0	Open	0
37	F	97	0	Open	0
38	M	97	Mild	Open	0
39	F	97	Mild	Open	0
40	F	97	0	Open	

Probably this was due to some other nutritive or metabolic influence, since the same phenomenon has been detected in a small percentage among all groups of apparently healthy animals.

The identity of the material deposited in the medullary canal is of interest. There was always present a variable amount of apatite, since it has been impossible, so far, to effect a complete separation of the original bone mineral and the new deposit. There is also the possibility that some bone salt may have become detached from the cancella and been incorporated in the new deposit.

In all films taken after the cortex had been ground away, there was visible the clear apatite pattern. But there was also present a pattern carrying prominently some of the lines characteristic of powdered egg shell ($CaCO_3$). These patterns were not clearly reproducible for publication, but Figure 5 shows a pattern from egg shell powder.

DISCUSSION

From this investigation, it appears that estrogen facilitates the deposition in the medullary canals of the femora of mice a mineral identifiable as $CaCO_3$. The presence of apatite may indicate simply incomplete separation of the deposit from the original bone, detachment of apatite fragments from the latter with migration into the new deposits, or new formation of apatite in situ. It seems unlikely at present that the latter process occurs, but the evidence is indirect as no experimental approach to the point is apparent at this stage. The calcium-phosphorus ratios of the bones of estrogen-treated mice indicated an increase of calcium carbonate so that the chemical analyses are compatible with the above observations. Furthermore, the calcium: phosphorus ratio of the medullary deposit tended to exceed that of the cortex (4).

Aside from this new deposit, however, the effect on the orderly physical structure of the cortices of the bones is very constant. As stated in earlier papers, it is very difficult to determine what force is involved in bringing about a change in the position of the unit crystal already laid down along the axis of the shaft. Because the crystalline orientation tends to follow the lamellar orientation which is presumably determined by the arrangement of the osteoblasts it is possible that the osteoblast or the definitive osteocyte determines the structure of the inorganic matrix. Influences exerted on osteocytes, osteoblasts, or undifferentiated cells capable of becoming osteoblasts may alter (1) the orientation of the cells and hence the crystalline orientation, or (2) alter the capacity of the cells to form the usual inorganic matrix, namely apatite. That it is a factor susceptible to influence by vitamin D, parathyroid hormone and estrogen now seems certain. That the relationship is non-specific is indicated by the fact that disorientation may result from general nutritional disturbances.

CONCLUSIONS

Examination of the femora of healthy mice by x-ray diffraction technic shows that the cortices are composed of crystals of apatite oriented in patterns identical with those previously found for rats.

Mice with pellets of estrone implanted subcutaneously showed a disorientation of the apatite crystals in the femoral cortices and in the progressive deposition of a disoriented mixture of calcium carbonate with variable amounts of apatite.

REFERENCES

GARDNER, W. U., AND C. A. PFEIFFER: *Physiol. Rev.* 23: 139. 1943.
REED, C. I., AND B. P. REED: *Am. J. Physiol.* 138: 34. 1942.
REED, C. I., AND B. P. REED: *Ibid.* 143: 413. 1945.
WENTWORTH, J. H., P. K. SMITH, AND W. U. GARDNER: *Endocrinology* 26: 61. 1940.

THE EFFECTS OF ALLOXAN DIABETES ON THE IN-VITRO OXIDATION OF SUBSTRATES BY RAT TISSUES[1]

ATTILIO CANZANELLI, RUTH GUILD, AND DAVID RAPPORT
From the Department of Physiology, Tufts College Medical School
BOSTON

WHILE the evidence indicates that in general tissues of depancreatized animals do not oxidize carbohydrate in-vitro, the observations of Baker, Fazekas, and Himwich (1938) indicate that in such animals glucose continues to be oxidized by the brain. The experiments reported in this communication were instituted primarily to see whether this was also true in the brain of animals given alloxan, which facilitates the study of severe diabetes in rats and other animals in whom complete removal of the pancreas is difficult. The effect of this form of diabetes on the oxidation of other substrates by brain, as well as by liver and kidney, was also studied, and a few observations were made on brain oxidation in phlorhizin diabetes. Albino rats of the Wistar strain were used, and the respiration of tissue slices observed in the Warburg manometric apparatus, the pH of the medium being kept within physiological limits during the experiments.

BRAIN RESPIRATION

a) *Alloxanized Rat.* Alloxan was injected subcutaneously in a single dose of 200 mgm. per kilo. The blood sugar was observed at intervals. At the end, usually of 48 hours, provided the animal was clearly diabetic, with an elevated blood sugar, it was killed by a blow on the head and the tissues studied. The blood sugar of animals fasted 18 hours was as high as 1400 mgm. per cent; the average in 33 animals was 600 mgm. per cent, about half the animals being fasted, the remainder getting food ad lib. It will be seen in Table I that when the brain was respiring in a modified phosphate-buffered Ringers solution ("MPBR"; composition: 0.143 M Na, 0.0047 M K, 0.0013 M Ca, 0.0006 M Mg, 0.010 M P, 0.131 M Cl, 0.006 M SO_4) the Q_{O_2} was 5.4 ± 0.1. In 33 experiments on brains taken from alloxan treated animals the Q_{O_2} was 6.9 ± 0.2. This statistically significant increased oxygen consumption of the diabetic brain in a substrate-free medium is not fully explained. Previous fasting does not affect it. It may be

Received for publication March 12, 1946.
[1] Aided by grants from the Committee on Research in Endocrinology of the National Research Council, and the Charlton Fund.

due to the fact that in the alloxanized animal the blood sugar, and presumably the glucose in the brain tissue, is higher than that in the normal animal. If in the diabetic brain glucose is capable of elevating the oxygen uptake, it is theoretically possible to account for the effect observed. For example, assuming that the diabetic brain cell has a concentration of 600 mgm. per cent glucose, it can be calculated that

TABLE I. THE Q_{O_2} OF TISSUES IN NORMAL AND DIABETIC RATS

	MPBR[1]	Glucose 0.005 M	Pyruvate 0.01 M	Lactate 0.01 M	Succinate 0.01 M	Glutamate 0.01 M
Brain						
Normal Rats	5.4±0.1 (50)	9.7±0.2 (39)	9.0 (4)	10.3 (4)	7.3±0.3 (4)	9.5 (4)
Alloxanized Rats	6.9±0.2 (33)	10.3±0.2 (25)	10.1 (3)	11.1 (3)	9.9±0.1 (4)	10.9 (3)
Phlorhizinized Rats	5.8±0.2 (8)	10.9±0.4 (8)				
Liver						
Normal Rats	11.1±0.6 (8)	9.7 (5)	14.0 (3)	15.5 (4)	21.5 (4)	10.9 (4)
Alloxanized Rats	9.3±0.6 (8)	9.7 (6)	14.5 (4)	15.0 (3)	22.3 (3)	11.8 (3)
Kidney						
Normal Rats	19.7 (6)	20.1 (6)	26.9 (3)	28.6 (3)	38.0 (3)	29.0 (3)
Alloxanized Rats	19.9 (5)	20.8 (5)	24.2 (3)	26.2 (3)	39.4 (3)	28.0 (3)

[1] Described in text.

respiration could go on for two to three hours at the expense of this extra amount of substrate, whereas the glucose in the normal brain cell would under the conditions of the experiment be exhausted during the preliminary manoeuvres before the measurement of oxygen consumption was begun. Actually, while the figures of the individual experiments show that brains of animals having the highest blood sugar tend to have the highest Q_{O_2}, the correlation is not a good one, and it can only be stated as a probability.

When 0.005 M glucose is added to the medium, the brain Q_{O_2} of the alloxanized animal rises to 10.3 and that of the normal animal to 9.7. It is clear therefore, that the brain of a rat made diabetic with alloxan can utilize glucose as well as the normal brain, and this is true even when its diabetes is so severe that the fasting blood sugar is as high as 1400 mgm. per cent. From Table I it will also be seen that

sodium pyruvate and lactate are also utilized as well by the diabetic as, and sodium succinate somewhat better than, the normal brain. Glycogen added to the medium is not oxidized by brain, but does not inhibit, by surface action or otherwise, the oxidation of added glucose.

Glutamic acid presents a specially interesting case. The fact that it is oxidized by brain was observed by Quastel and Wheatley (1932); Krebs (1935) and Weil-Malherbe (1936). We have confirmed these observations in the normal brain, and find that the diabetic brain oxidizes this amino acid as well as the normal brain does (Table I). Weil-Malherbe (loc. cit.) studied the effect upon brain oxygen consumption of thirteen amino acids, namely 1(+) glutamic acid, 1(+) alanine, 1(+) valine, 1(−) leucine, 1(−) proline, 1(−) hydroxyproline, dl-serine, 1(−) aspartic acid, 1(+) arginine, 1(−) tryptophane, 1(−) methionine, 1(+) ornithine, and 1(−) histidine. In view of the implications regarding carbohydrate metabolism involved, we have repeated these observations on all of the above amino acids except the last three, and have studied in addition the effects of glycine, dl phenylalanine, 1(+) lysine, dl isoleucine, and dl norleucine. We have fully confirmed and extended Weil-Malherbe's observations. The observed Q_{O_2}'s in our experiments follow: MPBR alone 5.4; alanine 4.3; aspartic acid 3.3; phenylalanine 3.3; leucine 3.7; glycine 4.2; arginine 4.6; proline 5.5; lysine 5.3; serine 4.4; tryptophane 4.7; valine 4.7; isoleucine 3.8; norleucine 4.2; hydroxyproline 5.4. There is a suspicion of inhibition in the case of some of these amino acids, especially aspartic acid and phenylalanine, but since the above are all averages of only 3 observations, it is not possible to speak with certainty concerning this. It is however clear that of all amino acids thus far tried, only glutamic acid appears to be utilized by brain, and this ability, as we have indicated above, is not impaired when the animal is made diabetic with alloxan. The fact that glutamic acid can be oxidized while aspartic acid and alanine, whose deamination products enter directly into the tricarboxylic acid cycle, apparently are not, may be due to an inability of the brain to deaminize any other amino acid than glutamic. Again, although histidine, arginine, citrulline, proline and hydroxyproline can form glutamic acid in liver or kidney (Krebs, 1943), presumably they cannot in the brain.

b) *Phlorhizinized Rat.* Eight rats were injected subcutaneously with 100 to 300 mgm. per kilo. of phlorhizin in oil for a period usually of 2 to 3 days before the experiments. The fasting blood sugar in these experiments was inconstant. In four experiments it was definitely elevated after amounts of phlorhizin that always result in low blood sugar in the dog. In three others it was normal. In one, phlorhizin was given for 6 days and the animal fasted, with a falling blood sugar that was finally 40 mgm. per cent. However, a fasting rat, even though diabetic will not infrequently have a low blood sugar. It seems likely that the renal effect of phlorhizin in the rat is slight, and is over-

shadowed by interference with sugar oxidation elsewhere. This subject requires further investigation. In any event, the oxidation of glucose by brain slices in the phlorhizinized rat is not impaired.

c) *Alloxan and Phlorhizin In-Vitro.* Alloxan has no effect in-vitro on the oxygen consumption of brain without substrate, or on its ability to oxidize glucose. Phlorhizin, on the other hand, inhibits both, reducing the Q_{O_2} without added substrate to 4.0 and with glucose to 7.2. The reason this occurs in-vitro and not when the whole animal has been phlorhizinized is probably a matter of differences in concentration, for as Shorr, Loebel, and Richardson have shown (1930), in 0.002 M concentrations, such as was used in these experiments, phlorhizin depresses cell respiration.

LIVER AND KIDNEY RESPIRATION

The results in Table I indicate that neither rat liver nor kidney in-vitro utilize added glucose in either the normal or the diabetic state. Liver does not oxidize added glutamate, while kidney does. Pyruvate, succinate, and lactate all increase the oxygen consumption of both tissues, and the ability to make use of these substrates is not affected at all by the diabetic state.

Alloxan added to the medium does not appreciably alter the respiration of liver (Q_{O_2} 10.9 ± 0.1); on the other hand, in line with the histological evidence of renal damage after the administration of alloxan, the oxygen consumption of kidney is reduced 10 to 15 per cent (av. $Q_{O_2} = 17.4$).

SUMMARY

Brain slices of rats made diabetic with alloxan, oxidize glucose, pyruvate, lactate, and succinate as well as does normal brain. Glutamic acid, alone of 18 amino acids, (our own data plus those of Weil-Malherbe) is oxidized by normal brain, and in our experiments is oxidized equally well by diabetic brain. The brain of the phlorhizinized animal oxidizes glucose. Liver and kidney of alloxanized and normal animals oxidize pyruvate, lactate, and succinate; added glutamate is utilized by kidney (diabetic and normal) but not by liver; added glucose by neither.

REFERENCES

BAKER, Z., J. F. FAZEKAS, H. E. HIMWICH: *J. Biol. Chem.* 125: 545. 1938.
KREBS, H. A.: *Biochem. J.* 29: 1620. 1935.
KREBS, H. A.: *Advances in Enzymology* 3: 245. 1943.
QUASTEL, J. H., AND A. H. M. WHEATLEY: *Biochem. J.* 26: 725. 1932.
SHORR, E., R. O. LOEBEL AND H. B. RICHARDSON: *J. Biol. Chem.* 86: 529. 1930.
WEIL-MALHERBE, H.: *Biochem. J.* 30: 665. 1936.

THE PASSAGE OF ENDOGENOUS ESTROGEN ACROSS THE PARABIOTIC UNION IN RATS[1]

ISOLDE T. ZECKWER, M.D.

From the Department of Pathology, University of Pennsylvania Medical School

WHEN an ovariectomized rat is united in parabiosis with a normal female rat, it is well known that FSH secreted by the pituitary of the ovariectomized rat soon passes over the parabiotic union and stimulates the ovaries of the second rat to secrete estrogen. Experiments reported previously (Zeckwer 1944) correlated the changes in the pituitary, mammary glands and ovaries of such rats over long periods up to 18 months after operation. In the late stages the pituitary, mammary glands and uterus of the ovariectomized partner (rat A) were of the castrate type, whereas the other partner (rat B) showed all the effects of long continued estrogen secretion. The parabiotic union was by suture of peritoneum, muscle and skin, but with no gross opening between the peritoneal cavities of the two rats. At the time of these experiments, it was not appreciated that at an early stage after parabiosis, estrogen passed from rat B to rat A temporarily so that there was a stage of estrus in the ovariectomized animal.

The present experiments describe the histological studies of the tissues during this temporary stage when both the ovariectomized rat A and its partner were found to present a curious phenomenon dependent upon the effect of progestin superimposed upon estrogen in B and of estrogen which had passed over to A from B. The results are described because: (1) they give information of the conditions under which a high level of estrogen in the blood stream occurs, and (2) they give information in regard to the conditions which determine vaginal mucification.

Methods. 19 pairs of litter-mate female rats were operated upon, while still immature, 32 to 59 days of age, 3 pairs at 61, 66 and 108 days of age. One of each pair (rat A) was ovariectomized at the same time. Vaginal smears of both partners were followed at frequent intervals, so that the rats could be killed at the desired stage. The time interval after operation studied was 9 to 91 days. Earlier and later time intervals in over a hundred pairs previously reported served for comparison. Single intact litter-mates and single ovariectomized litter-mates served as controls.

Received for publication March 25, 1946.

[1] Aided in part by a grant from the Faculty Research Committee of the University of Pa. Presented in abstract before the American Physiological Society, Federation Proceedings 5: 117. 1946.

Results. Soon after the operation, the ovarian follicles of B, stimulated by FSH from A, as is well known, secrete estrogen, resulting in the usual vaginal changes of cornification (fig. 2). Rat A at this time shows atrophic vaginal epithelium (fig. 1). Then follows the period which is the subject of the present experiments. This may begin as early as 11 days after operation or may not occur until many days later. The ovariectomized rat A develops cornified vaginal epithelium (fig. 3) proving that estrogen has passed to A from B. At the same time the cornified vaginal epithelium of B has been changed to mucification (fig. 4), as extensive as that seen in pregnancy. This passage of estrogen across the parabiotic union occurs at the time that the ovaries of B have developed many large corpora lutea (fig. 7) dependent upon estrogen causing the release of LH from the pituitary of B (Witschi and Levine 1934). This is also at the stage when estrogen releases from the pituitary of B a factor causing hyperplasia of the mammary gland (fig. 8). Occasionally secretion by the mammary acini begins at this stage. Although estrogen has passed to A, the mammary glands of A remain atrophic (fig. 9). Apparently estrogen in A inhibits the secretion of FSH by the pituitary of A, as evidenced by reduction in the castration cells in the pituitary of A. It is probably as a result of this, that various fluctuations occur in the vaginal epithelium of the 2 rats. Sometimes slight mucification occurs in A (fig. 13) sometimes cornification occurs in both rats, sometimes cornified smears in each rat disappear and reappear. Various modifications in changes occurred, as a combination of mucification and cornification in B (fig. 14). Eventually these fluctuations are succeeded by constant estrus in B (fig. 6) and atrophy in A (fig. 5) and (fig. 12). This occurs as early as 37 days after operation or slightly later, when all corpora lutea have disappeared, presumably because estrogen has exhausted the pituitary of B of LH or prevented further formation of LH. The ovary then develops cystic follicles (fig. 10) and the hyperplastic mammary acini are distended with secretion (fig. 11). Eventually the ovaries become a solid mass of granulosa cells which continue to secrete estrogen for the rest of the life of the rat, and simulates granulosa cell tumor of the ovary seen in women. In one

---

FIG. 1. Atrophic vaginal epithelium of ovariectomized rat A, 27 days after parabiosis. ×92.

FIG. 2. Cornification of vaginal epithelium of rat B united with ovariectomized rat illustrated in Fig. 1, 27 days after parabiosis. ×92.

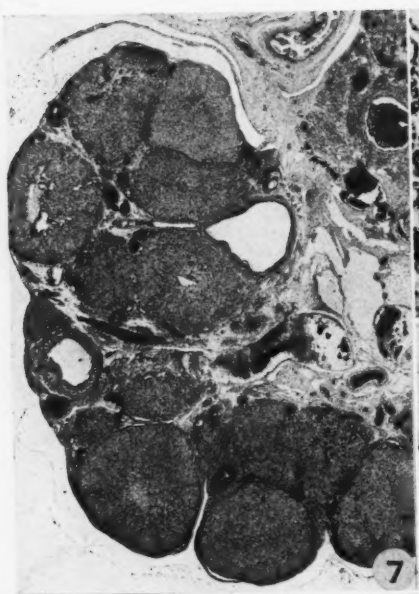
FIG. 3. Cornification of vaginal epithelium of ovariectomized rat A, 30 days after parabiosis. ×92.

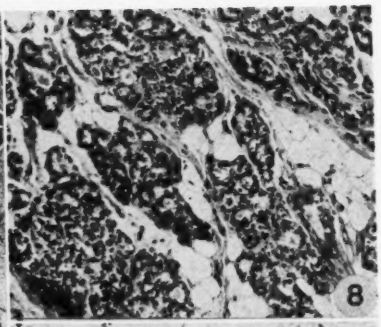
FIG. 4. Mucification of vaginal epithelium of rat B united to the ovariectomized rat illustrated in Fig. 3. ×92.

FIG. 5. Atrophic vaginal epithelium in ovariectomized rat A, 56 days after parabiosis. ×92.

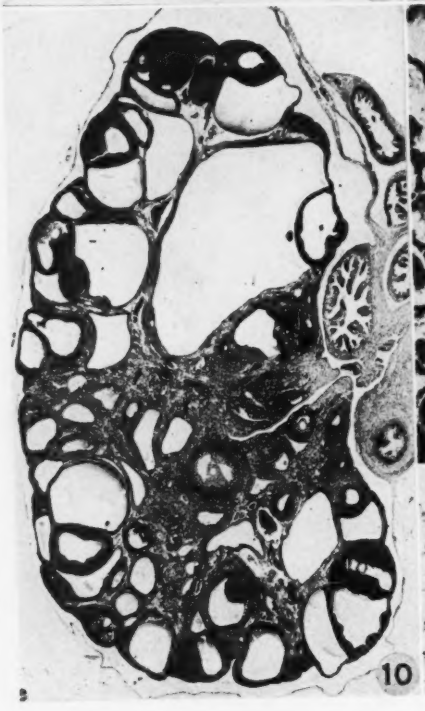
FIG. 6. Cornification of vaginal epithelium of rat B united with the ovariectomized rat A illustrated in Fig. 5. ×92.

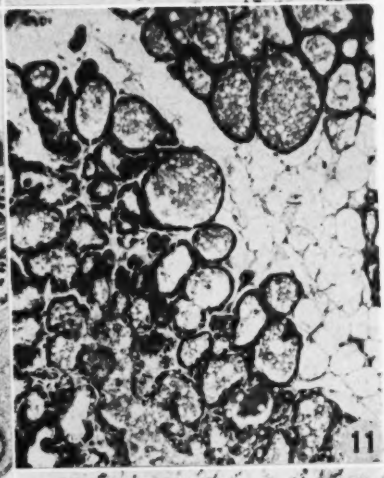
1
2
3
4
5
6

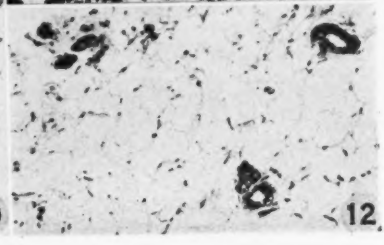

7


8

9


10


11


12

pair of rats in which estrus had appeared and disappeared 5 times in rat A, when they were killed at 42 days after operation, all corpora lutea had disappeared in B and cornification was present in A. This was the only exception to the general rule that when estrogen was known to pass to A corpora lutea in B were present.

DISCUSSION

The experiments indicate the conditions under which estrogen passes from B to A. Biddulph, Meyer and Gumbreck (1941) found that when exogenous estrogen was injected into one of a pair of parabiotic rats, it required 40 times the dosage effective in a single rat to pass across the circulation of the parabiotic union. Du Shane *et al.* (1935) found that daily injections of 100 rat units of estrin into one of a pair did not produce estrus in the ovariectomized partner, because estrin disappeared so rapidly from the circulation. On that basis, in the present experiments, at the time when estrogen passes across, it presumably is at a very high level in the blood. The experiments show that this high level coincides with the time of development of corpora lutea. It is only when corpora lutea disintegrate and disappear permanently that estrogen ceases to pass to A.

Data are available on the effect of progestin modifying the destruction and excretion of estrogen (Smith and Smith 1931; Pincus and Zahl 1937; Smith, Smith, and Pincus 1938). This subject is reviewed by Doisy, Thayer and vanBruggen (1942). Apparently in the present experiments any possible alterations in excretion or destruction of estrogen does not deplete the blood stream of estrogen when corpora lutea are present. That progestin in some way alters the estrogen so as to make it pass more easily to the other rat without a quantitative increase in the blood is a theoretical possibility, but one for which there is no evidence at present. Another theoretical possibility is that the utilization of estrogen is decreased in the presence of progestin, and consequently accumulates in the blood. Again no evidence for this is available.

The experiments indicate the conditions under which mucification of vaginal epithelium occurs. The significance of mucification of the

---

FIG. 7. Many large corpora lutea in one half the cross section of an ovary of rat B illustrated in Fig. 4 & Fig. 8. ×23.

FIG. 8. Hyperplasia of mammary gland in the rat B illustrated in Fig. 4 and Fig. 7. ×92.

FIG. 9. Atrophic mammary gland of ovariectomized rat A illustrated in Fig. 3. ×92.

FIG. 10. Cross section of the ovary of rat B illustrated in Fig. 6 and Fig. 11. Corpora lutea have disappeared. Cystic follicles and masses of granulosa cells. ×23.

FIG. 11. Secreting hyperplastic mammary gland of the rat B illustrated in Fig. 6 and Fig. 10. ×92.

FIG. 12. Atrophic mammary gland in ovariectomized rat A illustrated in Fig. 5. ×92.

vagina has been reviewed by Allen, Hisaw and Gardner (1939) and by Burrows (1945). Mucification is not regarded as a positive effect of progestin on vaginal epithelium, but as the effect of estrogen combined with progestin. Mucification also occurs when estrogen is combined with androgen, while androgen alone produces no histological change in vaginal epithelium. It may occur if cornifying doses of estrogen are injected into a rat which has active corpora lutea or if progestin in suitable dosage is injected with a cornifying dose of estrogen. According to Korenchevsky and Hall (1937) it occurs strikingly when the proportion is 1500γ progesterone to 1γ estrone. Selye, Browne and Collip (1936) produced it with 400γ progesterone to 30γ estrone. Jones and Astwood (1942), who review the literature on this subject, object to the effect of progestin being spoken of as an "inhibition" or "antagonism" to estrogen. In their experiments they obtained extensive mucification in an ovariectomized rat with 100μg. of estradiol and 1 mg. of progesterone daily for 11 days. They regard the absolute amount of progesterone as more important than the ratio of estrogen to progesterone. They regard mucification as the production by the two hormones of an entirely different type of histological change which can be obtained in the presence of high levels of estrogen if the dose of progesterone is adequate.

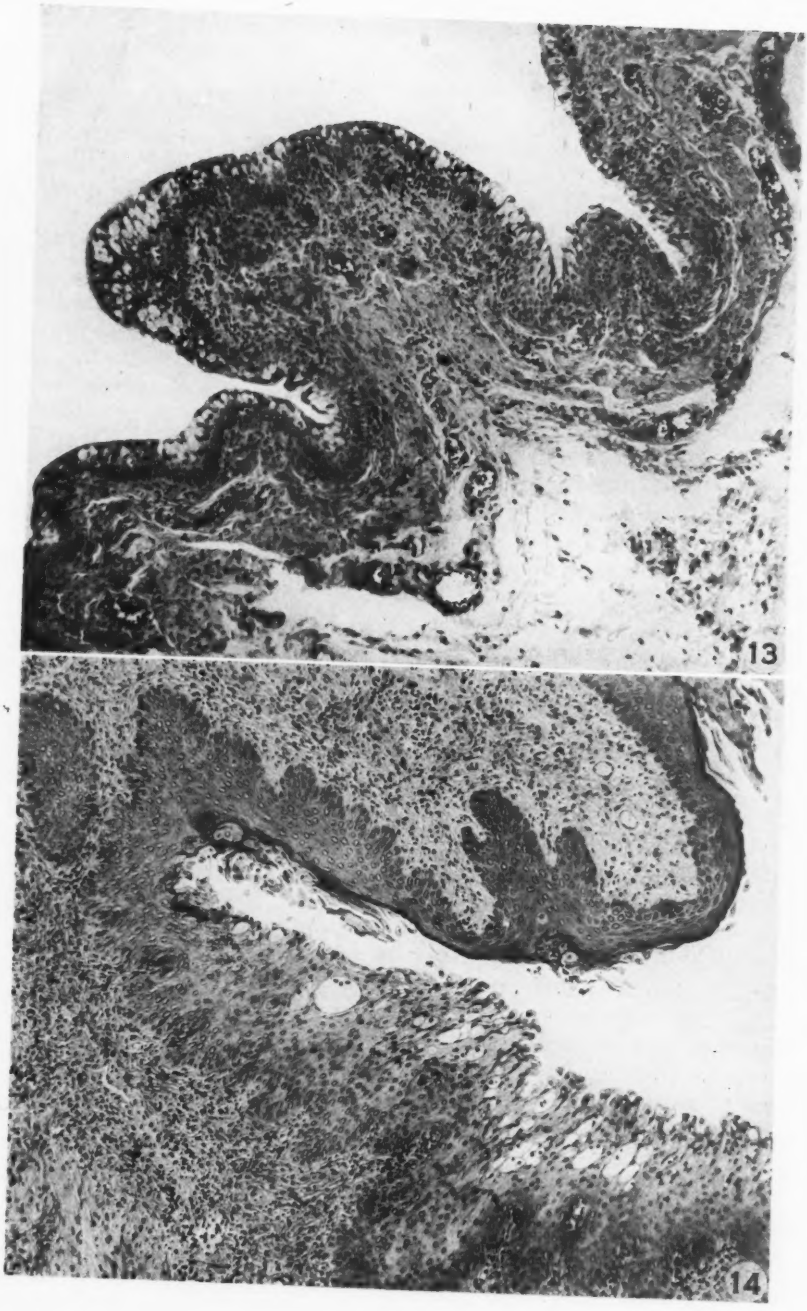
How progestin modifies the action of estrone on vaginal epithelium is not known, except that it is not by way of the pituitary. Apparently, in the present experiments it is not due to progestin causing a loss of estrogen by excretion in urine or bile, because we have seen that blood estrogen probably is at its highest point at this time. It seems unlikely that alterations in utilization of estrogen would account for the effect. Rather, the data suggest that the modification of estrogen by progestin takes place peripherally in the tissues that react to estrogen, as in the vaginal epithelium.

There is a difference of opinion as to whether mucification may also occur with estrogen alone in less than a cornifying dose, without the presence of progestin. Under these conditions vacuolated cells appear, the change is of only moderate degree and the layers of epithelial cells are not so numerous. The change is regarded by some (Selye, Browne & Collip 1936; Korenchevsky and Hall 1937) as not identical with the effect of estrogen being altered by progestin while many other workers make no distinction between these changes. Some of the ovariectomized rats showed this change (fig. 13). Other A rats showed a more extensive cellular vacuolization than in Figure 13 with development of squamous epithelium under the mucified

---

FIG. 13. Ovariectomized rat A 30 days after parabiosis showing slight mucification of vaginal epithelium. ×92.

FIG. 14. Rat B united with the ovariectomized rat A illustrated in Fig. 13, showing a combination of mucification and cornification of vaginal epithelium. ×92.


13
14

layer. It was not possible to decide whether this resulted from a small dose of estrogen reaching rat A, or whether it indicated that progestin. as well as estrogen had passed from B to A.

The data presented amplify the studies of previous investigators. That rats united with gonadectomized partners show a number of irregular cycles for some weeks before constant estrus is established was observed by Hill (1933), Witschi and Levine (1934), Du Shane, Levine, Pfeiffer and Witschi (1935) and others. At this stage of irregular cycles, corpora lutea were present according to Hill (1933), Witschi and Levine (1934), whereas after constant estrus had developed, corpora lutea were regressing or were absent according to Hill (1933). Witschi and Levine 1934 regarded the periods of anestrus in B as due to LH of rat B and thought that eventually FSH of rat A suppressed the release of LH from rat B. Emphasis had usually been placed on the changes in the rat united with the gonadectomized partner. Of those workers studying the changes in the castrated partner, Fels (1929) noted continuous diestrus in the castrated partner and Du Shane, Levine, Pfeiffer, and Witschi (1935) comment upon the castrate condition of the vagina and uterus of the castrated partner during the periods studied by them. Hill (1933) who gives charts of the vaginal smears in both partners, indicates that the ovariectomized rat was always found in diestrus. Martins (1929), however, in studying 2 pairs of rats found that the castrated rat developed estral smears, as estrus disappeared in the intact rat which had corpora lutea, and that estrus was sometimes present in both partners simultaneously. Pinto (1945) in studying a 15 day period of parabiotic rats in order to study the adrenals noted that estrus was found in these rats, in some cases in both animals.

SUMMARY

When an ovariectomized rat A is united in parabiosis with a normal female rat B, pituitary FSH from A stimulates the ovarian follicles of B to secrete estrogen. Estrogen causes release of LH from the pituitary of B. Estrogen passes from B to A, as evidenced by cornification of vaginal epithelium in A only at the stage when many large corpora lutea have developed in B. At this stage the blood estrogen appears to be at a high level. At the same time, in rat B, previous cornification of vaginal epithelium changes to mucification, due to progestin modifying the effect of estrogen. This apparently is not due to progestin neutralizing estrogen in the blood stream but to some peripheral effect on the tissues.

At a later stage when corpora lutea regress and completely disappear, rat B shows all the effects of continuous secretion of estrogen but no estrogen passes to rat A. This failure of estrogen to pass across the parabiotic union at this time may be because the level of blood estrogen is not sufficiently high in the absence of corpora lutea.

REFERENCES

ALLEN, E., F. L. HISAW, W. U. GARDNER AND E. ALLEN: "Sex and Internal Secretions" —2nd Edition, Williams & Wilkins Co., Baltimore, 1939, p. 475.

BIDDULPH, C., R. K. MEYER AND L. G. GUMBRECK: *J. Exper. Zool.* 88: 17. 1941.

BURROWS, H.: Biological Actions of Sex Hormones. Cambridge University Press. 1945.

DOISY, E. A., S. A. THAYER AND J. T. VAN BRUGGEN: *Federation Proceedings* 1: 202. 1942.

DU SHANE, G. P., W. T. LEVINE, C. A. PFEIFFER, AND E. WITSCHI: *Proc. Soc. Exper. Biol. & Med.* 33: 339. 1935.

FELS, E.: *Med. Klin.* 25: 557. 1929.

HILL, R. T.: *Endocrinology* 17: 414. 1933.

JONES, G. E. S., AND E. B. ASTWOOD: *Endocrinology* 30: 295. 1942.

KORENCHEVSKY, V., AND K. HALL: *J. Path. & Bact.* 45: 681. 1937.

MARTINS, T.: *Compt. rend. Soc. de biol.* 102: 605, 614. 1929.

PINCUS, G., AND P. A. ZAHL: *J. Gen. Physiol.* 20: 879. 1937.

PINTO, R. M.: *Am. J. Physiol.* 144: 652. 1945.

SELYE, H., J. S. L. BROWNE AND J. B. COLLIP: *Proc. Soc. Exper. Biol. & Med.* 34: 198, 472. 1936.

SMITH, G. V., AND O. W. SMITH: *Am. J. Physiol.* 98: 578. 1931.

SMITH, G. V., O. W. SMITH AND G. PINCUS: *Am. J. Physiol.* 121: 98. 1938.

WITSCHI, E., AND W. T. LEVINE: *Proc. Soc. Exper. Biol. & Med.* 32: 101. 1934.

ZECKWER, I. T.: *Arch. Path.* 38: 99. 1944.

NOTES AND COMMENTS

ACID-BASE EQUILIBRIUM IN RELATION TO THE MENSTRUAL CYCLE

Our interest was aroused in this problem by the reports of Greenhill and Freed (1941) and Freed (1945); they administered ammonium chloride for the relief of premenstrual tension or distress with remarkable success. An increase in extracellular fluid, dependent on a retention of sodium, was thought to be a responsible factor in the production of the symptoms which preceded the onset of the menses. No blood analyses were reported, and we have been unable to find complete information regarding the acid-base equilibrium of the blood in relation to the menstrual cycle. It was thought that if there is a retention of sodium preceding the menses, it might be evident as an increase in total base of the serum.

Not many investigators have studied total base values. In 1926, Peters *et al.* gave the range of total base as 147 to 161 millimols in the serum of normal individuals. Consolazio and Talbott (1940) give the range of total base as 147.7 to 156.8 m.eq./l. in a series studied by them. Eckstein, Lubran and McKeown (1940–41) studied certain chemical changes in the blood in relation to the menstrual cycle, but did not include total base.

Since we could not find the information we desired, we decided to investigate the following: pH (glass electrode), carbon dioxide content (Van Slyke manometric method), chloride (open Carius method) and total base (resin method, Polis and Reinhold, 1944). A group of 13 women who suffered from the symptoms of premenstrual tension volunteered to act as subjects. Twenty-two determinations were made at the midpoint between two periods when the subjects felt well, and 19 were made during the premenstrual tension, when the subjects felt miserable, just before the onset of the menses. The results are shown below; the chloride and total base are given in m.eq./l.; the carbon dioxide content is given both in m.eq./l. and in volumes per cent.

	Midpoint		During tension	
	Range	Average	Range	Average
ph	7.31–7.42	7.34	7.29–7.41	7.36
CO_2	25.2–31.5	27.9	26.1–30.6	27.9
	(56–70)	(62)	(58–68)	(62)
Cl	94–103	100	97–105	100
Total base	150–162	159	149–164	158

Since the differences in the serum constituents determined were so small between the midpoint and the period of premenstrual tension, we thought it advisable to study a group of women who had no difficulty with menstruation to see if the differences were of the same order of magnitude. Nine subjects were studied; 18 determinations were made at the midpoint and 17

Received for publication February 21, 1946.

either just before or immediately after the onset of the menses. The results are shown below.

	Midpoint		At the time of the menses	
	Range	Average	Range	Average
pH	7.27–7.45	7.36	7.28–7.47	7.36
CO_2	25.2–31.1	28.8	23.4–31.5	27.9
	(56–69)	(64)	(52–70)	(62)
Cl	95–104	99	96–105	102
Total base	152–162	157	150–163	155

Fasting blood sugar derminations were done on this group also since the influence of menstruation on carbohydrate metabolism is still a moot point (Cramer, 1942; Tedstrom, 1936). It was found that the blood sugar just at the onset of the flow was slightly lower than at the midpoint.

Finally, we thought it worth while to study the fluctuations in a group of men, in order to note the magnitude of changes which occurred when not influenced by the menstrual cycle. Thirty-five determinations were made on 10 male medical students, at intervals of two weeks. The results are shown below.

	Range	Average	Individual variations	Average difference during the six weeks
pH	7.30–7.40	7.35	0.03 to 0.1	0.05
CO_2	27.9–35.6	32	0.9 to 5	2.3
	(62–79)	(71)	(2 to 11)	(5)
Cl	93–105	99	0.55 to 7.92	4
Total base	152–173	160	2 to 15	7

SUMMARY

In the women studied there was no constant change in pH, carbon dioxide content, chloride or total base in relation to the menstrual cycle, either in those who suffered premenstrual tension or in those who were free from menstrual difficulties. We concluded that the menstrual cycle is not characterized by typical serum changes in acid-base equilibrium.

In the men studied the carbon dioxide content was higher than in the women; the fluctuations in pH, carbon dioxide content, chloride and total base over a six week period were as great or greater than in women.

ESTHER M. GREISHEIMER, M. J. OPPENHEIMER AND DOROTHY ELLIS
From the Department of Physiology,
Temple University School of Medicine,
Philadelphia, Pennsylvania

REFERENCES

CONSOLAZIO, W. V., AND J. H. TALBOTT: *J. Biol. Chem.* 132: 753. 1940.
CRAMER, H. I.: *Canad. M.A.J.* 47: 51. 1942.
ECKSTEIN, F. M. P., M. LUBRAN AND T. MCKEOWN: *Guy's Hosp. Rep.* 90: 28. 1940–41.
FREED, S. C.: *J.A.M.A.* 127: 377. 1945.
GREENHILL, J. P., AND S. C. FREED: *J.A.M.A.* 117: 504. 1941.
PETERS, J. P., H. A. BULGER, A. J. EISENMAN AND C. LEE: *J. Biol. Chem.* 67: 141. 1926.
POLIS, B. D., AND J. G. REINHOLD: *J. Biol. Chem.* 156: 231. 1944.
TEDSTROM, M. K., AND L. E. WILSON: *California & West. Med.* 44: 375. 1936.

A NOTE ON THE ABSENCE OF ANDROGEN FROM A DIET OF NATURAL FOODS

The androgen content of natural foodstuffs is usually disregarded in hormone assays. Koch[1] states that there is no evidence that changes in diet affect the excretion rate of the sex hormones. However, in the course of studies on dietary factors affecting the adrenal, we wished to assay the stock diet for a possible androgen content.

The standard diet of natural foodstuffs, Purina Chow, has been compared with a "synthetic" diet in regard to its effect on the rat prostate. Weanling rats, at one month of age, were castrated under ether anesthesia, and placed

THE EFFECT OF DIET ON BODY GROWTH AND ORGAN WEIGHTS IN CASTRATED RATS

Diet	No. of rats	Final body wt. gms.	Body wt. gain gms.	Adrenal wt.	Seminal vesicle wt.	Ventral lobe of prostate
				Body wt. $\times 10^{-5}$	Body wt. $\times 10^{-5}$	Body wt. $\times 10^{-5}$
Chow	8	121.4	60.1	1.89	7.24	8.03
B	7	144.7	80.7	1.71	6.53	7.04

half on a diet of chow, half on a synthetic diet (B) of the following percentage composition: casein 12, starch 39, dextrin 26, dried yeast 8, Osborne and Mendel's salt mixture 3, wheat germ oil 1, cod liver oil 1, inert solids (cellu flour) 10. After 3 weeks on the diets, the rats were killed with ether, and the tissues dissected and weighed. The data are given in the table. The larger gain in body weight on the synthetic diet is statistically significant. The slight differences in the organ weights are not statistically significant.

It is concluded that the Purina Chow did not contain appreciable amounts of orally active androgens.

RICHARD S. BENUA AND EVELYN HOWARD

From the Department of Physiology,
Johns Hopkins University School of Medicine,
Baltimore, Maryland

Received for publication February 27, 1946.

[1] Koch, F. C.: *Harvey Lec.* 33: 231. 1938.

THE EFFECT OF THYROIDECTOMY ON THE TISSUE RESPONSE TO ADMINISTERED THYROXIN

Quite a few observations have been made in regard to the effect of injecting thyroxin or feeding dessicated thyroid on the oxygen consumption of isolated tissues. While they vary considerably, on the whole they indicate that thyroid medication tends to increase the metabolism of certain tissues. It is not our purpose to review these observations, but to point out that previous thyroidectomy renders the differences, as observed in rat liver, sufficiently large and constant to make the method of some value as a "spot test" for either thyroid preparations or their inhibitors.

Received for publication March 7, 1946.

The animals used were both male and female, about 6 months old, weighing between 200 and 250 gm. The oxygen uptake was determined on slices in the Warburg apparatus. In the thyroidectomized animals the operation was performed 4 to 6 weeks prior to the experiment. d-1 Thyroxin, 20 mgm. per kilo., was injected subcutaneously 48 hours before the experiment in a single dose. The results are seen in the table. The Q_{O_2} of normal livers was 10.5 ± 0.59; that of livers from thyroxin injected rats was 12.7 ± 0.64, an increase of 21 per cent. While this is statistically significant, the margin is not large. The Q_{O_2} of the liver of thyroidectomized animals was 8.3 ± 0.41, or 21 per

Q_{O_2} OF TISSUES AFTER THYROXIN ADMINISTRATION

	Normal	Thyroxin Injected	Increase	Thyroidectomized	Thyroidectomized thyroxin injected	Increase
			%			%
Liver	$10.5+0.59(9)$[1]	$12.7 \pm 0.64(12)$	21	$8.3 \pm 0.41(15)$	$12.3 \pm 0.49(15)$	48
Kidney	$19.7 \pm 0.77(6)$	$23.1 \pm 0.78(12)$	21	$17.6 \pm 0.72(15)$	$21.3 \pm 0.74(15)$	23

[1] Figures in parentheses denote number of experiments.

cent less than the normal animal; after thyroxin, it rose to 12.3 ± 0.49, representing an increase of 48 per cent. This difference is unmistakable, for the value $\bar{x}/\sqrt{\delta_1^2+\delta_2^2}$ where $\bar{x}$ is the difference in Q_{O_2} and δ is the standard deviation of the mean, is 6.2, which makes the possibility of the difference being a random one negligible. In the case of the kidney, while previous thyroidectomy decreases the O_2 consumption, it does not apparently sensitize the tissue to the effects of thyroxin.

DAVID RAPPORT, ATTILIO CANZANELLI, AND RUTH GUILD
From the Department of Physiology
Tufts College Medical School
Boston, Massachusetts